



BULLETIN OF PHARMACEUTICAL SCIENCES

Assiut University
ISSN - 1110-0052

Bulletin of Pharmaceutical Sciences Assiut University

[Home](#) [Browse](#) [Journal Info](#) [Guide for Authors](#) [Submit Manuscript](#) [Contact Us](#)

[Login](#) [Register](#)

Editorial Board



Editor-in-Chief

Prof. Gihan Fetih

Vice Dean of Faculty of Pharmacy

Professor, Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Egypt

gfetih@aun.edu.eg



Associate Editor

Prof. Dina Fathalla Mohamed Mohamed

Head of Department of Pharmaceutics

Professor, Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Egypt

dina.mohamed@pharm.aun.edu.eg



Executive Editor

Prof. Jelan Abd Elrazik Abd Elalim Abd Elrazik

Professor, Industrial Pharmacy Department, Faculty of Pharmacy, Assiut University, Egypt

jelan.abdelrazik@pharm.aun.edu.eg



Editorial Board

Prof. Soad Abdel latief Hassan Bayoumi

Head of Department of Pharmacognosy

Professor, Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Egypt

soad.bayoumi@pharm.aun.edu.eg



Prof. Ahmed Safwat Mohamed Aboraia

Head of Medicinal Chemistry Department
Professor, Medicinal Chemistry Department, Faculty of Pharmacy, Assiut University, Egypt
✉ ahmed.mohamed15@pharm.aun.edu.eg



Prof. Noha Nahedj Atia Mohamed

Professor and Head of Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assiut University, Egypt
✉ noha.mohamed1@pharm.aun.edu.eg



Prof. Hesham Mohamed Tawfeek Hassan

Professor and Head of Pharmaceutical Industry Department, Faculty of Pharmacy, Assiut University
✉ heshamtawfeek@aun.edu.eg



Prof. Mohamed N. Seleem

Tyler J. and Frances F. Young Chair in Bacteriology Professor
Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA
✉ seleem@vt.edu

Explore Journal

- [Home](#)
- [About Journal](#)
- [Editorial Board](#)
- [Submit Manuscript](#)
- [Contact Us](#)
- [Glossary](#)
- [Hard Copy Subscription](#)
- [Sitemap](#)



Latest News

Newsletter Subscription

Subscribe to the journal newsletter and receive the latest news and updates

✉ [Subscribe](#)





 Volume & Issue: Volume 47, Issue 2, December 2024, Pages 681-1470 

Number of Articles: 50

RECENT ADVANCES OF B-BLOCKERS AS DRUG REPURPOSING APPROACH: A SYSTEMATIC REVIEW

Pages 681-698

Moaz A. Eltabeeb; Raghda Rabe Hamed; Mohamed A. El-Nabarawi; Mahmoud H. Teaima; Menna M. Abdellatif

View Article  PDF 1.01 M  View on SCiNiTO

DEVELOPMENT OF SERICIN HYDROGEL INCORPORATED WITH GREEN SYNTHESIZED CERIUM OXIDE NANOPARTICLES USING SECURINEGA LEUCOPYRUS FOR CHRONIC DIABETIC WOUND HEALING APPLICATIONS

Pages 699-721



Sriram k; Priyanka G; Dhanushri TN; udhayadharshini A

View Article  PDF 1.87 M  View on SCiNiTO

EXPLORING HERBAL NUTRACEUTICALS: UNLOCKING POTENTIAL IN NAUSEA AND VOMITING PREVENTION - A COMPREHENSIVE REVIEW

Pages 723-749



Marzooka Kazi-Chishti; Waleed Hussain Syed; Nazimuddin Chishti

View Article  PDF 1.11 M  View on SCiNiTO

THE ELIXIR OF YOUTH: RETINOID REVELATION

Pages 751-773

Sneha Nawale; Jeslina Roshni Benadict; Chenna Harshan Reddy Yenugula; Jagadish Swamy M; Monika Nijhawan

View Article  PDF 1.17 M  View on SCiNiTO

ELASTIC VESICLES FOR ENHANCING THE TRANSDERMAL DELIVERY OF OLMESARTAN MEDOXOMIL

Pages 775-788



reem Al-Uobody; Thair L. Jabbar; amira amin

View Article  PDF 1.14 M  View on SCiNiTO

DEVELOPMENT AND EVALUATION OF CURCUMIN-EUDRAGIT MICRO MICROSPONGES FOR ANTI-INFLAMMATORY THERAPY OF COLON

Pages 789-803

PREETHI SUDHEER; Syed Mohasin Abbas

View Article  PDF 1.99 M  View on SCiNiTO

BOX BEHNKEN MODEL-BASED LIQUISOLID COMPACT TECHNIQUE FOR THE IMPROVEMENT OF SOLUBILITY AND DISSOLUTION RATE OF AZELNIDIPINE

Pages 805-821



Dr. Abhishek Yogeshwar Kanugo; Rutik Jukti

View Article  PDF 1.57 M  View on SCiNiTO

PREPARATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES PHOSPHOLIPID COMPLEX LOADED WITH FENTICONAZOLE NITRATE FOR TOPICAL MANAGEMENT OF CANDIDA ALBICANS

Pages 823-835



Sarmad Dheyaa Noori; Hussein Hamed Hayal; Hayder Hammoodi Mohamed; Amira Amin

View Article  PDF 1.13 M  View on SCiNiTO

GC-MS ANALYSIS AND EVALUATION OF ANTIOXIDANT, ANTICANCER, CYTOTOXICITY, AND HYPOGLYCEMIC PROPERTIES OF THE LIBYAN MORINGA OLEIFERA LEAF EXTRACT ON ALLOXAN-INDUCED DIABETES IN MICE

Pages 837-852



Osama Abdel-Hafeez Mohamed Al-Bedak; Ahlam Rashed; Seham T.M. Oshkondali; Nouri Kushlaf; Amal Elshebani; Samira A. Alacrouk; Fadia T.M. Oshkondale; Manal Elsheli; Zuhur R. Almesai; Ameerah Shaeroun; Steven L. Stephenson; Fuad Ameen

View Article  PDF 1.45 M  View on SCiNiTO

ESTIMATION OF ANTIOXIDANT AND ANTITHROMBOTIC EFFECTS OF CRUDE OLEUROPEIN EXTRACTED FROM OLEA EUROPAEA USING THREE DIFFERENT TECHNIQUES

Pages 853-865



Naglaa M. Hamdy; Atheer Obaid; Ashwaq T. Hameed; Hoda S. Sherkawy; Hanan M. El-Tantawy; Shaimaa H. Sayer

View Article  PDF 867.23 K  View on SCiNiTO

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF FRACTIONS OF PINUS BRUTIA AND CEDRUS LIBANI LEAVES ETHANOLIC EXTRACTS

Pages 867-882



Arwa Abdelghafar Albakour; Ream Nayal; Mohammad Yaser Abajy

[View Article](#)  PDF 953.18 K  [View on SciNiTO](#)

OPTIMIZATION OF EXTRACTION PROCESS TO MAXIMIZE PHENOLIC CONTENT, FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF PROSOPIS FARCTA USING RESPONSE SURFACE METHODOLOGY

Pages 883-898



Mona Hamwi; Ream Nayal; Mohammad Yaser Abajy

[View Article](#)  PDF 982.79 K  [View on SciNiTO](#)

PHENOLIC CONTENT, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF HYDROMETHANOLIC EXTRACT OF LIMNOSPIRA FUSIFORMIS TL03 ISOLATED FROM TELAMINE LAKE, NORTHWEST ALGERIA

Pages 899-916



Guenachi Belkacem; Korteby Mefti Hakima; Lamari Lynda

[View Article](#)  PDF 1.03 M  [View on SciNiTO](#)

DEVELOPMENT OF AYURVEDIC FORMULATIONS FROM LEAVES OF CARICA PAPAYA AND COMPARISON OF PLATELET AUGMENTATION POTENTIAL AGAINST CYCLOPHOSPHAMIDE-INDUCED THROMBOCYTOPENIA

Pages 917-926



Divya Jyothi; Himanshu Joshi; Ullas Prakash Dsouza; kadeejath Rizwana

[View Article](#)  PDF 722.89 K  [View on SciNiTO](#)

DESIGN, SYNTHESIS, AND BIOLOGICAL ACTIVITY PROFILING STUDY OF IMIDAZOLINONE-BASED HYDRAZONES AS POTENTIAL MULTITARGET ANTIMYCOBACTERIAL AGENTS

Pages 927-952



Enas Taha Abdelhamed; ndia M Mahfouz; Farghaly Abd El Hameed Omar; Yasser Musa Ibrahim; Ahmed Megahed Abouwarda

[View Article](#)  PDF 1.53 M  [View on SciNiTO](#)

CHEMICAL COMPOSITION OF ESSENTIAL OILS, OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION AND EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF ANAGYRIS FOETIDA

Pages 953-965



Hamza ALIBOUDHAR; Zineb LAKACHE; Daouia Keltoum Benmaarouf; Nacéra TIGRINE-KORDJANI

[View Article](#)  PDF 972.83 K  [View on SciNiTO](#)

ACETYLCHOLINESTERASE, TYROSINASE AND PEROXIREDOXIN ENZYME INHIBITORY ACTIVITY STUDIES OF SYNTHESISED OXADIAZOLES AND THIADIAZOLES FROM SCHIFF BASES

Pages 967-987



JAINAY P JAMES; keerthan P shettigar; Sindhu T J; Zakiya F C

View Article  PDF 1.78 M  View on SCiNiTO

COMPUTATIONAL SELECTION AND OPTIMIZATION OF RNA APTAMER AGAINST ALPHA-FETOPROTEIN-AN IN SILICO STUDY

Pages 989-1008



Muhamad Muhamad alkriz; Dima joujeh

View Article  PDF 1.79 M  View on SCiNiTO

STUDYING MOLECULAR MODELLING OF THE CHOLESTEROL ABSORPTION INHIBITOR EZETIMIBE AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

Pages 1009-1023



Manila Abd alkarem Mohammed; Saleh Trefi; Mustapha Fawaz Chehna; Yaser Bitar; Ali Ibrahim

View Article  PDF 1.52 M  View on SCiNiTO

RSM-BASED OPTIMIZATION OF AN RP-HPLC METHOD, ANALYTICAL METHOD VALIDATION, AND CONTENT DETERMINATION OF DAIDZEIN IN SOY SAUCE

Pages 1025-1036



Florentinus Dika Octa Riswanto; Jovan Tri Wibowo; Emerentiana Rica Estri Wulanjari; Ketrin Sadna Martyas; Michael Raharja Gani; Sudibyo Martono

View Article  PDF 980.89 K  View on SCiNiTO

DEVELOPMENT AND VALIDATION OF GREEN HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF DAPAGLIFLOZIN AND LINAGLIPTIN IN COMBINED DOSAGE FORM

Pages 1037-1048



Dhyey Shah; Dhananjay Meshram; Dhara Patel; Prasanna Pradhan; Grishma Patel

View Article  PDF 823.05 K  View on SCiNiTO

SPECTROPHOTOMETRIC DETERMINATION OF MELOXICAM IN PURE FORM AND ITS PHARMACEUTICAL PREPARATIONS VIA OXIDATIVE COUPLING REACTION

Pages 1049-1062



Ali Mohammed Atiyah; Asmaa Ahmed Mohammed; Abdul Majeed Khorsheed Ahmed

View Article  PDF 1.28 M  View on SCiNiTO

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF CABOTEGRAVIR AND RILPIVIRINE USING RP-HPLC

Pages 1075-1063



Sahitya Bharathi Chodimella; Raja Sundararajan

View Article  PDF 752.36 K  View on SCiNiTO

GC-MS ANALYSIS OF SOME ENDOPHYTIC FUNGAL EXTRACTS

Pages 1077-1091



Abdullah H. Al-Saadoon; Najwa M. Jameel; Eman H. Bishara

View Article  PDF 963.28 K  View on SCiNiTO

Detection and Quantification of Cyproheptadine and Dexamethasone as Adulterants in Herbal Medicines and Dietary Supplements Marketed for Weight Gain

Pages 1093-1103



Arwa Ahmed Alyas; Hasan Saad Aldewachi; Mohammed Ibrahim Aladul

View Article  PDF 778.45 K  View on SCiNiTO

COMPARATIVE EFFICACY OF INTRAVENOUS MIDAZOLAM, MIDAZOLAM-KETAMINE, AND KETAMINE IN REDUCING THE PREVALENCE OF POST-SPINAL SHIVERING: A DOUBLE-BLIND CLINICAL TRIAL

Pages 1105-1117



Siavash Sangi; Nasim Alipour; Nahid Manouchehrian; Maryam Aligholizadeh

View Article  PDF 840.62 K  View on SCiNiTO

EXPLORING THE POTENTIAL HEPATOPROTECTIVE EFFICACY OF LEPIDIUM SATIVUM SEEDS: A COMPREHENSIVE REVIEW

Pages 1119-1135



Eman. S Sawan; Alaa A. Abdelhadi; Kareem H. Elsyed; Nora H. Elsayed; Heba R. Yassen; Manar R. Mohammed; Nadeen. S. Ahmed; Manar K. Zahran; Nada M. Elkest; Omar Hammad; Shimaa Shimaa Abdelsattar; Raghda Hussein; Mohamed M. Abdel-Latif; Shaza H. Aly

View Article  PDF 982.12 K  View on SCiNiTO

AN UPDATE ON VARIOUS COUNSELING APPROACHES FOR IMPROVING ASTHMA MANAGEMENT

Pages 1137-1151



Basma Mohamed; Saleh Alghamdi; Mohamed AbdElrahman; Nabila Laz; Hoda Rabea; Haitham Saeed; Mohamed Abdelrahim

[View Article](#)  PDF 795.34 K  [View on SciNiTO](#)

PREVALENCE OF POLYPHARMACY AMONG LIBYAN ELDERLY ADULTS

Pages 1153-1161



Ahmed Atia; Lama Ben Garsa; Fardous Jumma

[View Article](#)  PDF 622.67 K  [View on SciNiTO](#)

ENHANCING COMPLIANCE AND ADHERENCE OF INHALATION DEVICES AMONG ASTHMATIC PATIENTS: A COMPREHENSIVE REVIEW AND META-ANALYSIS OF INTERVENTIONS

Pages 1163-1175



Mohamed Abdelrahim; Hasnaa Osama

[View Article](#)  PDF 708.55 K  [View on SciNiTO](#)

NILOTINIB VS IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA-CHRONIC PHASE

Pages 1177-1188



Aiat Morsy Mohamed Morsy; Hoda Hassan Essa; Abeer Amin; Jean George Maxwell; Doaa Ali Gamal

[View Article](#)  PDF 617.19 K  [View on SciNiTO](#)

RECEPTOR FOR THE ADVANCED GLYCATION END PRODUCTS (RAGE) PROMOTER GENE POLYMORPHISMS IN INFLAMMATORY BOWEL DISEASE

Pages 1189-1202



Noha Refaat Abdelhamid; Elham Abdelsamie ALi; Dalia Tarik Kamal; Abeer Ahmed Mokhtar; Mohammed Ahmed Medhat

[View Article](#)  PDF 882.29 K  [View on SciNiTO](#)

NEURONAL GROWTH REGULATOR-1 (NEGR1) AND BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF): NOVEL TARGETS OF COMBINED FENOFIBRATE AND PIOGLITAZONE TREATMENT OF OBESITY INDUCED DEPRESSION IN RATS

Pages 1203-1214



Mona K Tawfik; Dahlia I Badran; Fatma Saad Samman

[View Article](#)  PDF 867.91 K  [View on SciNiTO](#)

POSSIBLE EFFECT OF VITAMIN D SUPPLEMENTATION ON METHOTREXATE ADVERSE EFFECTS IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

Pages 1215-1233



Mohamed Mostafa Elbadr; Magy Ibrahim Anis Rizkallah; Amira Mahmoud Osman; Mahmoud Hamdy Abdel-Raheem; Nivin Abdel-Azim Abdel-Rehim Hassan

[View Article](#)  PDF 1.43 M  [View on SCiNiTO](#)

ANTIOXIDANT EFFECTS OF ESOMEPRAZOLE, CURCUMIN, CHITOSAN AND CURCUMIN-CHITOSAN MIXTURE ON GASTRIC ULCER IN FEMALE RATS : EXPERIMENTAL STUDY

Pages 1235-1249



jenan Al-kawaz; Hussein Jasim Al-Harbi; Fakir magtoof Al-zubaidy

[View Article](#)  PDF 1.12 M  [View on SCiNiTO](#)

CYMBOPOGON CITRATUS: A MODULATOR OF TNF- α AND IL-10 IN NSAID-INDUCED HEPATORENAL TOXICITY IN ADULT WISTAR RATS

Pages 1251-1262



Akinpelu MORONKEJI; Temidayo D Adeniyi; Abiodun Oyeleke; Chuks M Igunbor; Joy Olajumoke AYENI; Toluwanimi Olanike ADELABI; Adebimpe Moronkeji

[View Article](#)  PDF 929.12 K  [View on SCiNiTO](#)

ALLEVIATING REPRODUCTIVE DYSFUNCTION OF LEAD ACETATE IN MALE RAT: THE ROLE OF CURCUMIN AND METFORMIN

Pages 1263-1272



Muhsin S.G Al Moziel; Abbas Abdulrazzaq Khudhair; Mohammed Azeez Qathee

[View Article](#)  PDF 910.88 K  [View on SCiNiTO](#)

INCRETIN-BASED THERAPY AND CANCER: ARE THEY ENEMIES OR ALLIES?

Pages 1273-1295



Sandy R. Botros; Asmaa Matouk; Gehan Heeba

[View Article](#)  PDF 916.51 K  [View on SCiNiTO](#)

THE PROTECTIVE EFFECTS OF TOCOTRIENOLS AND COENZYME Q10 AGAINST GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN RATS: POTENTIAL ROLES OF NRF2, BCL-2, AND AUTOPHAGY

Pages 1297-1310



Alaa T. Abdelhafez; • Asmaa M.S. Gomaa; Asmaa M. Ahmed; Manal M. Sayed; • Tohamy Anwar Tohamy; • Mai Assem Abd_Elkareem Ali; Sally A. Sayed

[View Article](#)  PDF 1.34 M  [View on SCiNiTO](#)

ANNONA MURICATA LEAF EXTRACT AMELIORATES HAEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES IN TESTOSTERONE-INDUCED MALE WISTAR RAT MODEL OF BENIGN PROSTATIC HYPERPLASIA

Pages 1311-1325



Amanda Ezirim; Josephine Ezekwesili-Ofili; Innocent Igwilo

View Article  PDF 1.03 M  View on SCiNiTO

DYSREGULATION OF EZRIN EXPRESSION AND PHOSPHORYLATION IN UTERINE LEIOMYOMA

Pages 1327-1339



khaled Mohammed Hassan yousef; Abdel-Raheim Mohamed Meki; Ayat Abdel-Rahman Sayed; Muhammad Maghraby; Tahia H Saleem

View Article  PDF 614.02 K  View on SCiNiTO

THE CONTRIBUTION OF INNATE IMMUNE CELLS TO THE PATHOPHYSIOLOGY OF ACUTE STERILE INFLAMMATION- EVIDENCE FROM PRECLINICAL ANIMAL MODELS

Pages 1341-1352



Nardeen Eldafashi; Shaimaa waz; Maiiada H. Nazmy; Moustafa Fathy

View Article  PDF 521.45 K  View on SCiNiTO

INVESTIGATION OF ANTICANCER MECHANISMS OF A CHALCONE/XANTHINE HYBRID IN NON-SMALL CELL LUNG CANCER

Pages 1353-1366



Omar Mahmoud Elrehany; Al - Shaimaa Faissal Ahmed; Hesham A. Abou-Zied; Alaa M. Hayallah; Mohamed Abdel-Aziz; Maiiada Hassan Nazmy

View Article  PDF 887.13 K  View on SCiNiTO

MICROBIAL ENZYMES IN LIPID METABOLISM: UNVEILING THE KEY PLAYERS IN HEALTH AND DISEASE

Pages 1367-1378



khaldoon Jasim Mohammed; Hadeel Ahmed kenoosh; Mariam Akeel

View Article  PDF 506.94 K  View on SCiNiTO

IMPACT OF ENERGY DRINKS ON KIDNEY TISSUE AND OXIDATIVE STRESS IN FEMALE WISTAR RATS AND THEIR NEONATES

Pages 1379-1392



Demiana H. Hanna; Heba AA El-Rahman; Aya ragab abdelghany; Mohamed A. A. El Desouky

[View Article](#)  PDF 1010.72 K  [View on SCiNiTO](#)

EFFECT OF LYCOPENE AND ANTHOCYANIN ON LIVER AND KIDNEY FUNCTIONS IN MALE AND FEMALE ALBINO RATS TREATED WITH DEXAMETHASONE

Pages 1393-1417



Sawsan Mohamed Kilany; Sara Mohamed Mahmoud; Mahmoud Abdelrazek Doheim; Safwat Hassan Ali

[View Article](#)  PDF 1.28 M  [View on SCiNiTO](#)

ANTIFUNGAL SUSCEPTIBILITY AND COMPARATIVE ANALYSIS OF BIOFILM PRODUCTION AND METABOLIC ACTIVITY IN CANDIDA SPP. ISOLATED FROM CLINICAL SAMPLES

Pages 1419-1430



Amel Benmessaoud; Wassim YEZLI; Mohamed Ouassini BENSaid; Sidahmed SAADI; Samira NAIR

[View Article](#)  PDF 746.38 K  [View on SCiNiTO](#)

ANTIBACTERIAL POTENTIALS OF SOME PLANTS EXTRACTS AND THEIR COMBINATIONS AND THEIR SYNERGISTIC ACTIVITIES AGAINST MULTIDRUG RESISTANT BACTERIA

Pages 1431-1446



suhair shaker AL-siraj; jihan mostafa Badr; Ashraf A. Abd El-Tawab

[View Article](#)  PDF 958.86 K  [View on SCiNiTO](#)

ANTIOXIDANT AND ANTICANCER ACTIVITY OF ACTIVE SECONDARY METABOLITES PRODUCED BY STREPTOMYCES GRISEORUBENS

Pages 1447-1458



Abdel-Hamied Rasmey; Merna Elgendy; Rania Ahmed; Aida Hussein

[View Article](#)  PDF 700.53 K  [View on SCiNiTO](#)

INCIDENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI CAUSING COMMUNITY-ACQUIRED URINARY TRACT INFECTION IN TIARET PROVINCE, WESTERN ALGERIA: RETROSPECTIVE STUDY

Pages 1459-1470

Rachid Merati; Abdellatif Boudra; Amina Sarah Maachi; Kheira Chams El Assala Messaoud

[View Article](#)  PDF 663.68 K  [View on SCiNiTO](#)



RSM-BASED OPTIMIZATION OF AN RP-HPLC METHOD, ANALYTICAL METHOD VALIDATION, AND CONTENT DETERMINATION OF DAIDZEIN IN SOY SAUCE

Florentinus Dika Octa Riswanto^{1,2*}, Jovan Tri Wibowo¹, Emerentiana Rica Estri Wulanjari¹, Ketrin Sadna Martyas¹, Michael Raharja Gani¹, Sudibyo Martono¹

¹*Division of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta 55282, Indonesia*

²*Research Group of Computer-Aided Drug Design and Discovery of Bioactive Natural Products, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta 55282, Indonesia*

Soybean is extensively consumed in Indonesia and is known to benefit human health. One of the soybean products is soy sauce. Soybean and its products are reported to contain isoflavone aglycone daidzein which can provide beneficial biological activities for humans. Hence, it is important to develop a suitable analytical method to analyze the daidzein content in soy sauce. The Box–Behnken design (BBD), a widely used response surface methodology (RSM), was performed to optimize the independent variables such as mobile phase composition of methanol and acetonitrile and flow rate condition. Analytical method validation including linearity and range, selectivity, accuracy, and precision was evaluated in this study. The optimized conditions for daidzein analysis were the mobile phase of methanol:water:acetonitrile (60:35:5 v/v/v), flow rate of 1.0 mL/minute, and detection wavelength at 251 nm. This condition met the requirements of the system suitability test with low variance for tailing factor, resolution, retention time, number of theoretical plates, and area under curve. The analytical method was successfully validated for several parameters including linearity and range, selectivity, accuracy, and precision. A reversed-phase high-performance liquid chromatography (RP-HPLC) method for analyzing daidzein in soy sauce can be optimized by implementing the Box–Behnken Design. This method met the analytical method validation acceptance criteria for linearity, range, selectivity, accuracy, and precision. According to the quantitative determination result, it was found that daidzein content in soy sauce sample was of 0.014 mg/g.

Keywords: daidzein, response surface methodology, reverse phase HPLC, soy sauce, validation

INTRODUCTION

Soybean (*Glycine max* L.) is known as a food material that contains high nutrients in Indonesia. Soybean is also reported to be beneficial for human health, such as reducing the risk of coronary heart disease and breast cancer¹. Soybean and soybean products are reported to contain many bioactive compounds². Isoflavones, one of the bioactive compounds group contained in soybean, are flavonoid subgroup with a 3-phenyl chromone structure³. The aglycone isoflavones in soybeans consist of daidzein, genistein, and

glycitein². As one of soybean products, soy sauce is quite popular in the Asian region including Indonesia and usually made through traditional fermentation processes⁴.

Fermentation in the soy sauce manufacturing process is divided into two stages: the *koji* fermentation stage and the *moromi* fermentation stage. This fermentation process has an impact for isoflavone content in soy sauce. The concentration of isoflavone glycosides is very high in the *koji* fermentation stage, but gradually decreases and is replaced by aglycone isoflavones in the *moromi* fermentation stage. This is because the

fermentation process involves the hydrolysis of isoflavone glycosides into aglycone isoflavones⁴. Daidzein (**Fig. 1**) as one of isoflavone aglycones is a non-steroidal phytoestrogen compound with potential antioxidant activity. Some biological activities of daidzein include cardioprotective, anticancer, and osteoporosis prevention effects⁵.

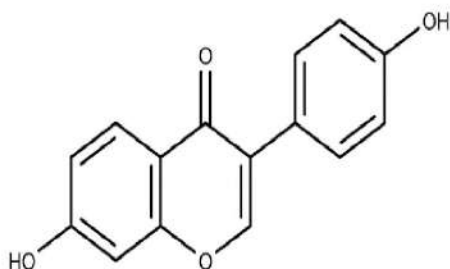


Fig. 1: Chemical structure of daidzein.

One of the analytical methods used for the separation of daidzein is a reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC was used due to its process automation, relatively fast, and efficient⁶. HPLC is a separation technique that employs a solid stationary phase and a liquid mobile phase⁷. The optimization of the analytical method using HPLC can be assisted by applying the Response Surface Methodology (RSM). Response surface methodology can be conducted in the optimization research equipped by a free statistical computation purpose namely R software⁸. The RSM model of the Box-Behnken Design (BBD) can be generated to assist the optimization⁹.

Previous study from Yuliani *et al.* (2018) reported the extraction optimization to achieve genistein and daidzein from tempeh, a fermented product from soybean¹⁰. Other studies reported the evaluation of daidzein along with genistein and glycitein in soy milk products^{8, 11}. However, there are still limited studies reporting the daidzein evaluation in soy sauce marketed in Indonesia. Since it was reported that daidzein has several benefits to human health, it is important to provide nutritional information related to the isoflavone aglycone in soy sauce.

The study aimed to implement the RSM-based optimization of RP-HPLC method along with the analytical method validation for

determining the content of daidzein in soy sauce samples. In this study, methanol percentage, acetonitrile percentage, and flowrate were selected as independent variables or factors, whereas several chromatographic responses including resolution, retention time, tailing factor, and theoretical plate number were stated as dependent variables or responses.

MATERIAL AND METHODS

Material

Daidzein and genistein reference standards were bought from Sigma-Aldrich, Singapore. The solvents that utilized were liquid chromatography grade of methanol (Merck Millipore, Germany), ethyl acetate, acetonitrile (Merck Millipore, Germany), petroleum ether (Smart Lab, Indonesia), and redistilled water that prepared in Laboratory of Faculty of Pharmacy, Universitas Sanata Dharma, Indonesia. Sodium sulfate anhydrous was bought from Merck Millipore, Germany. Soy sauce with the brand of "ABC" was purchased from a local market in Sleman, Yogyakarta, Indonesia.

Instruments and Application

Instrumentation utilized in this study were HPLC system of Shimadzu® LC-2010 CHT (Japan) with UV/Vis detector and a computer, a C18 column of Hibar® 250-4,6 Purospher® STAR (Germany) RP-18 endcapped (5 µm), ultra-micro analytical balance RADWAG® (Poland) series of UYA 2.3Y (max: 2.1 g, min 0.01 mg), an Orbital Shaker, a system of Buchi Rotary Evaporator (UK), Retsch® T460 ultrasonicator (Germany), Gast® vacuum pump model DOA-P504-BN (USA), sterile syringe filter with a 0.2 µm pore size hydrophilic PTFE membrane (Merck Millipore, Germany), a set of Socorex® (Switzerland) micropipettes, and glasswares. R software version 4.1.3 and RStudio software version 4.1.3 with a rsm package were used to run the application of statistical technique and response surface.

Preparation of Standard and Sample Solution

Approximately 1.0 mg daidzein and 1.0 mg genistein were weighted and transferred into a different 5 mL volumetric flask. The stock solution for each standard with concentration 200 µg/mL was prepared by

diluting the content of each volumetric flask with methanol. The mixture solution containing daidzein and genistein standard was obtained by transferring 0.5 mL of both standard stock solutions into the same 5 mL volumetric flask, continued by dilution with methanol into the volume. The solution then filtered using a sterile syringe filter membrane (0.45 μm) before being injected into the HPLC system.

The sample preparation method was adapted with several modifications from the previous study⁸. Fifty milliliters of soy sauce and 100 mL petroleum ether were added into an Erlenmeyer with the ratio of sample and petroleum ether of 1:2 (v/v). Liquid-liquid extraction was carried out for 40 minutes (150 rpm) using orbital shaker and covered with an aluminium foil. Then petroleum ether was removed to obtain an extract of soy sauce.

Fifty milliliters of ethyl acetate were added into an Erlenmeyer that containing the extract which was prepared. Then followed by extraction process with orbital shaker for 90 minutes (150 rpm). Liquid-liquid extraction was carried out by adding 50 mL distilled water in separatory funnel. This process was repeated until three times. The filtrate of ethyl acetate was transferred into beaker glass. Sodium sulfate anhydrous was added to the solution followed by filtration process to obtain a yellow-coloured solution of ethyl acetate. Dry extract was obtained by evaporating the yellow-coloured solution using rotary evaporator. The obtained dry extract was diluted with 10 mL methanol.

Response Surface Methodology

RSM was applied in this study to optimize HPLC separation. Mobile phase used in this study were methanol, acetonitrile, and redistilled water. Box Behnken Design has been applied to analyze three independent variables such as the percentage of methanol (X1), the percentage of acetonitrile (X2), and

flowrate (X3) with three different level each. Every independent variable was coded as -1, 0, +1 for low, medium, and high levels, respectively. The variables and their observed values for each level are shown in **Table 1**. HPLC separation responses including tailing factor (Y1), resolution (Y2), retention time (Y3), and theoretical plate number (Y4) opted as dependent variables. The BBD design used in this study was acquired through the application of this formula utilizing R software:

```
> library(rsm)
> bbd(3, randomize=TRUE)
```

BBD model with 16 runs that generated using R software was used to perform each run by HPLC system of Shimadzu LC-2010 CHT with UV/Vis detector and a C₁₈ column of Hibar 250-4,6 Purospher STAR RP-18 uncapped (5 μm). Wavelength detection was set to 251 nm and the injection volume of each run was 10 μL .

Multiple Response Optimization

The results from 16 runs using BBD were documented for each response to construct RSM model. The impact of independent variables on each dependent variable was suited using the RSM function, applying a second-order model. The statistical analysis involved an analysis of variance (ANOVA) table to identify significant variations among the independent variables. Statistical analysis using R software also showed stationary points and eigenvalues that help the optimization process. Perspective plots for each dependent variable were also built by R software to visualize RSM model. Multiple response optimization was also performed since there were four different dependent variables with contrasting goals (maximum or minimum) for each variable. The desirability function was applied in this multiple-response optimization process. This computational optimization was done using R software.

Table 1: Levels of variables and their observed values

Variables	Low (-1)	Medium (0)	High (+1)
X1: Methanol (%)	60	65	70
X2: Acetonitrile (%)	5	10	15
X3: Flowrate (mL/min)	0.6	0.8	1.0

System Suitability Test

The system suitability test was conducted for both reference standard and soy sauce sample. A mixture solution containing all standards with a concentration of 20 µg/mL for daidzein and genistein each was injected to the system. The reference standard solution was injected to the system with injection volume of 10 µL and replicated six times.

The solution of sample was diluted by taking 1.0 mL sample solution and inserted to 10 mL volumetric flask. The daidzein reference standard solution was taken 2.5 mL with concentration of 20 µg/mL, inserted to the same volumetric flask and diluted with methanol to achieve a sample solution. The sample solution of 10 µL was injected to the system and replicated six times.

Analytical Method Validation

Selectivity. The standard daidzein solution with a concentration of 20 µg/mL was taken 0.5 mL and transferred into 5 mL volumetric flask, then methanol was added into the volume. The similar procedure was done to prepare a standard genistein solution of 20 µg/mL. After that, 2 mL of the extracted sample was taken and then transferred into 5 mL volumetric flask, added with methanol into the volume. Then 1.5 mL was taken from each volumetric flask, filtered with filter membrane, and transferred into different HPLC vial. The selectivity test was done by identifying the peak profile. The accepted standard for resolution value was more than 1.5 which demonstrate good peak separation.

Sensitivity. Sensitivity of the method was evaluated using the limit of detection (LOD) and limit of quantitation (LOQ) value. The LOD and LOQ were determined using standard deviation approach according to Miller & Miller¹². The LOD and LOQ can be calculated using the following formulas:

$$LOD = y_B + 3s_B \dots\dots\dots (1)$$

$$LOQ = y_B + 10s_B \dots\dots\dots (2)$$

where y_B is the analyte concentration giving a signal equal to the blank signal and s_B is standard deviation of the blank.

Linearity and range. Solutions for this test were prepared from stock solution at ten concentration levels (1–60 µg/mL). A calibration curve was created by plotting the concentration versus the peak area. The correlation coefficient, slope, and intercept were determined.

Accuracy and precision. Solutions for accuracy and precision test were obtained by transferring 100 µL (0.1 mL) of the sample solution combined with the standard daidzein solution at three different concentration levels (20, 35, and 50 µg/mL) into 10 mL volumetric flask for each concentration and diluted with methanol. Each solution was injected 10 µL into the HPLC system and wavelength detection at UV-251 nm. Accuracy and precision were evaluated by calculating the recovery percentage and Relative Standard Deviation (RSD) values at three concentration levels (low, medium, high) with three replicates for each concentration.

Content Determination of Daidzein

In this study, 2.0 mL of the sample solution was transferred into 10 mL volumetric flask, then diluted with methanol. The sample was filtered using 0.45 µm millipore filter membrane, then transferred to HPLC vial. Injection was replicated six times; the AUC value was recorded and the levels were calculated by plotting the AUC value against the standard curve. The average value of levels and its RSD were determined.

RESULTS AND DISCUSSION

Results

Response Surface Methodology

A set of sixteen runs of experiment were carried out with different combination of X1, X2, X3 using BBD with four central point's replication and generated into second order response surface methodology. These runs enable to collect responses data of dependent variables Y1, Y2, Y3, Y4. After successfully collect the responses data, the empirical models of RSM were generated using R software.

A predictive model has a significant effect on response if the p-value is <0.05 (5%)⁸. The independent variable can be stated to significantly affect the response if the multiple $R^2 \geq 0.8$ and adjusted $R^2 > 0.8$, with the difference between the multiple R^2 and adjusted R^2 values ≤ 0.2 ⁸. The lack of fit value from the model obtained must >0.05 so it can be stated that the regression model is suitable (there is no lack of fit)¹³. Excluding the tailing factor, all equation models generated met the acceptable p-value ($p < 0.05$), both multiple R^2 and adjusted R^2 , and lack of fit with $p > 0.05$.

Table 2: The Box Behnken Design and the results obtained from daidzein separation using RP-HPLC method.

Run	Factors			Responses			
	Methanol (%)	Acetonitrile (%)	Flow rate (mL/min)	Tailing factor	Resolution	Time retention	Theoretical plate number
1	65	10	0.80	1.30	2.13	4.76	4908
2	70	10	0.60	1.31	1.28	5.77	5367
3	70	15	0.80	0.00	1.72	4.07	5139
4	65	10	0.80	1.33	0.22	4.81	4901
5	60	10	0.60	1.34	3.27	7.13	5356
6	60	10	1.00	1.29	2.37	4.34	4338
7	60	5	0.80	1.29	4.38	6.51	5057
8	65	15	0.60	1.36	2.18	5.79	5758
9	65	5	1.00	1.29	3.14	4.44	4355
10	65	5	0.60	1.34	3.29	7.25	5419
11	65	10	0.80	1.32	0.70	4.82	5067
12	60	15	0.80	1.33	2.53	4.73	5147
13	70	5	0.80	1.32	0.90	4.89	5033
14	65	10	0.80	1.29	1.11	4.77	4728
15	65	15	1.00	1.29	0.72	3.47	4237
16	70	10	1.00	1.28	0.77	3.50	4157

Table 3: RSM analysis of tailing factor, resolution, retention time, and theoretical plate number.

Responses	p-value	Multiple R ²	Adjusted R ²	Lack of Fit	Stationary points (<i>eigen values</i>)		
					Methanol (%)	Acetonitrile (%)	Flow rate (mL/min)
Tailing factor	0.22	0.745	0.363	1.25e-04	63.97 (4.15)	8.56 (0.2e-03)	0.82 (-0.01)
Resolution	0.04	0.867	0.667	0.75	70.09 (10.48)	10.01 (0.04)	0.87 (0.01)
Retention time	1.67e-09	0.999	0.999	0.37	64.73 (7.23)	16.46 (0.01)	1.19 (0.2e-03)
Theoretical plate number	3.26e-04	0.977	0.941	0.81	59.53 (7.71)	6.081 (1.25)	0.43 (-3104.36)

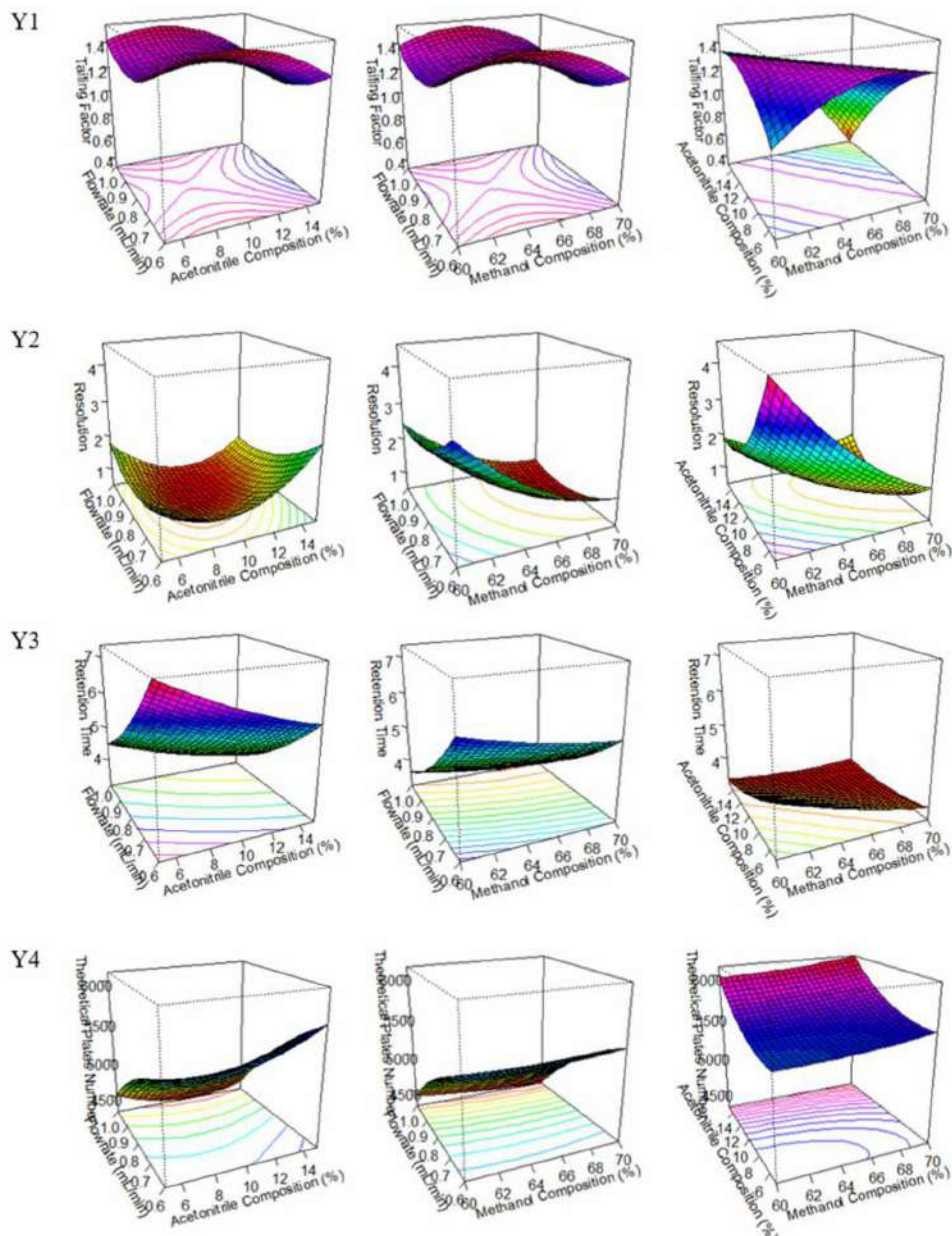


Fig. 2: The perspective plot of tailing factor (Y1), resolution (Y2), retention time (Y3), and theoretical plate number (Y4) for daidzein.

Stationary points and eigen values for each independent variable were generated by R software. The stationary points generated from the R application are not used further because in several cases the stationary points are estimates that cannot be applied to research⁸. This can be seen in the estimated flow rate on the number of theoretical plates response which was too slow and in the retention time response which was too fast. If the flow rate was too slow, it led to longer analytical times and potential band broadening. In the other hand, a

faster flow rate increased the column pressure and potentially shortening the column's lifetime⁸. The eigenvalues characterized the pattern of the perspective contour plot. Positive eigenvalues represented an upward curve, while negative eigenvalues depicted a downward curve. Therefore, all negative eigenvalues denoted a maximum stable point, all positive eigenvalues signified a minimum stable point, and mixed-sign eigenvalues suggested a saddle-shaped stable point lifetime⁸. All the results of the perspective contour plot that were

successfully formed from each dependent variable can be seen in **Fig. 2**. It can be observed that responses of tailing factor (Y1), resolution (Y2), retention time (Y3), and theoretical plate number (Y4) for daidzein can be affected by independent variables of flowrate vs acetonitrile composition, flowrate vs methanol composition, and methanol composition vs acetonitrile composition. The maximum stable point resulted from the negative eigenvalue of the model, whereas the minimum stable point resulted from the positive eigenvalue. The mixed sign of eigenvalue (positive and negative) indicated the saddle profile of the stable point⁸.

Daidzein can be separated using reversed-phase HPLC with C18 column because of the possibility of interaction between isoflavone aglycone daidzein with the silica-based column^{10, 14}. Analysis of daidzein compound in soy sauce using reverse phase HPLC with a mixture of methanol, water, and acetonitrile as mobile phase. Methanol was chosen because it can interact with the isoflavone aglycone daidzein through hydrogen interactions where daidzein can act as a hydrogen donor or acceptor⁸. Acetonitrile (CH₃CN) has very good solubility in water and water has polar properties so that both can be used as mobile phases in reverse phase HPLC^{15, 16}.

Multiple Response Optimization

Three out of four responses that have the value of p-value, R-square, and lack of fit that meet the requirements was selected. The three response models chosen were resolution, retention time, and theoretical plate number and were chosen to build a predictive model for daidzein separation in HPLC. The purpose of retention time response is to obtain the lowest value, while the purpose of theoretical plate number and resolution response are to obtain the highest value. Therefore, it is necessary to perform multiple response optimization. Multiple response optimization was carried out

by calculating the desirability function in R software. The desirability function has a value from 0 to 1. A value of 0 indicates the most unwanted response because the response is outside the range of the target value and a value of 1 indicates the most desirable response because the response is close to the target value^{17, 18}. The individual desirability function will be calculated by entering the maximum desirability formula in R software with the target for each response as shown in **Table 4**.

Table 4

The individual desirability function will form the total desirability function which is the geometric mean of the individual desirability functions¹⁸. The value of the total desirability function was obtained for the three separation responses and this value will be used as a reference for determining the optimum condition for daidzein analysis using HPLC. This function was executed in software R using the following formula:

$$D = (d1 \times d2 \times d3)^{1/3} \dots\dots\dots (3)$$

where,

D = Total desirability

d1 = individual desirability retention time

d2 = individual desirability resolution

d3 = individual desirability theoretical plate number¹⁸

The total desirability value of each combination of the three independent variables is calculated using software R and the combination that has the best total desirability value is taken. Based on the results, the optimum conditions for daidzein analysis using HPLC were the mobile phase composition of methanol:water: acetonitrile (60 : 35 : 5 v/v/v) and a flow rate of 1 mL/minute with a total desirability value of 0.57. This optimum condition will then perform a system suitability test.

Table 4: Target of Multiple Response Optimization with Desirability Function.

Responses	Limit		Target
	Lower	Upper	
Retention time	3	7	Minimum
Resolution	1.5	5	Maximum
Theoretical plate number	4300	5000	Maximum

System Suitability Test

System suitability test is an inseparable part of the analysis when using high performance liquid chromatography method. This test aims to ensure that the chromatography system is adequate for carrying out the analysis and provides results that are close for each test⁷. The system suitability test was carried out by running six injections for both of the standard mixture of daidzein and genistein, also the soy sauce sample. Genistein standard solution was also injected in this study to evaluate the separation between two isoflavone aglycones. The results of parameters such as tailing factor, resolution, theoretical plate number, and retention time were evaluated. System suitability test results for both reference standard and soy sauce sample are presented in **Table 5**.

Chromatogram profiles of system suitability test were presented in **Fig. 3**.

From the test result of the reference standard, all parameters met the acceptance criteria, such as the relative standard deviation of AUC and retention time $\leq 2\%$, resolution more than 2, tailing factor value ≤ 2 , and the value of the number of theoretical plates more than 2000¹⁹. Parameters that meet the criteria indicate that the reverse phase HPLC system is adequate and can provide results that are close to each other.

From the test results of the soy sauce sample, almost all parameters met the acceptance criteria, such as the relative standard deviation of AUC and retention time $\leq 2\%$, resolution did not meet the acceptance criteria ($R_s > 2$), tailing factor value ≤ 2 , and the value of the theoretical plate number more than 2000¹⁹.

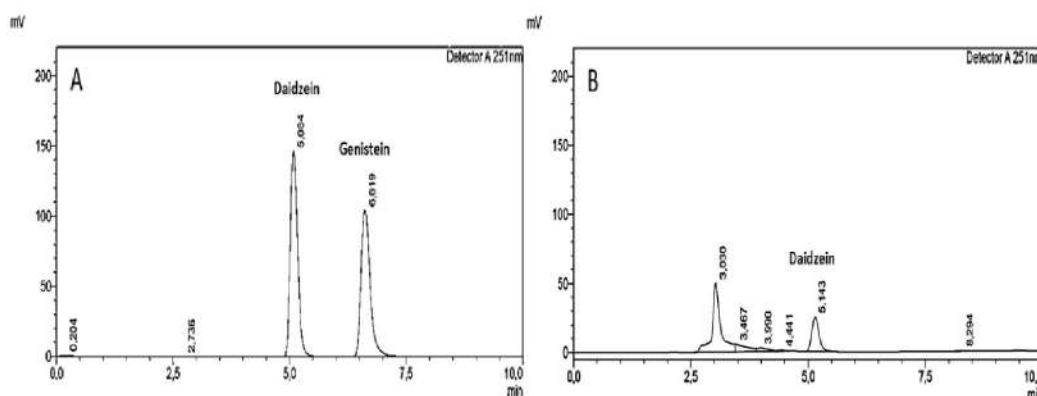


Fig. 3: HPLC chromatograms of mixture standard solution containing daidzein and genistein (A) and soy sauce sample solution containing daidzein (B). Mobile phase: methanol, water, acetonitrile (60:35:5). Flowrate: 1 mL/min. Column: C18 column of Hibar® 250-4,6 Purospher® STAR RP-18 endcapped (5 μ m) at UV-251 nm. Volume injection: 10 μ L.

Table 5: Results of system suitability test (n = 6).

Solution	Tailing factor	Resolution	Theoretical plate number	Retention Time		Area	
				Mean	RSD (%)	Mean	RSD (%)
Standard solution	1.245	9.155	4894.167	5.090	0.151	1609658	0.418
Soy sauce sample solution	1.269	1.623	5409.833	5.181	0.534	268127	1.009

Analytical Method Validation

Selectivity

Selectivity is an indicator that shows the extent to which a method can measure certain analytes in a complex mixture without interference from other components in the mixture. Selectivity can be seen by separating adjacent peaks by calculating the resolution value (R_s) (Sahoo et al., 2018). Separation can be said to be selective if the value of $R_s \geq 1.5^{21}$. This stage was carried out by injecting four different solutions, namely blank, daidzein standard, mixed daidzein and genistein standard, and soy sauce extract samples. The standard peaks produced are used to be compared with the peaks produced in the sample solution, so that it will be easy to find out the identity of the daidzein compound peak in the soy sauce extract sample. While the blank, is used to determine that the solvent used does not affect the reading of the desired compound. Chromatogram profiles of selectivity evaluation were presented in Fig. 4.

Linearity and range

The linearity range used in this test for daidzein were 1.01 - 60.44 $\mu\text{g/mL}$. Calibration curve equation of daidzein were $y = y = 55139x + 4984.60$ ($r = 0.9978$). These results showed that the method used in this test can provide a linear response to daidzein concentrations within the range.

Accuracy and precision

Accuracy and precision of the method was determined using standard addition method in three levels of concentration (low, medium, high) with three replications from each concentration. According to Table 6, the mean value of the recovery as the accuracy parameter at three concentration levels of daidzein are within the required range. Percentage of RSD as the precision parameter at three levels of daidzein were below the maximum limit of AOAC's requirement. These results show that this method produced highly precision and accuracy for determining daidzein in all concentration levels.

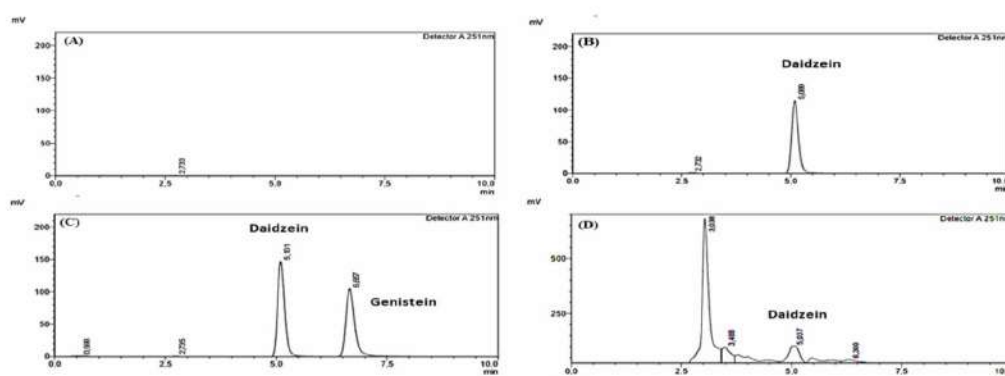


Fig. 4: Solvent blank (methanol) (A), HPLC separation profiles of daidzein standard (B), daidzein and genistein standard mixture (C), and soy sauce sample (D). Column: C18 column of Hibar® 250-4,6 Purospher® STAR RP-18 endcapped. Wavelength detection at 251 nm. Volume injection: 10 μL .

Table 6: Accuracy and Precision Result of Spiked Soy Sauce Samples (n=3).

Intraday					
Compounds	Level	Added amount ($\mu\text{g/mL}$)	Found amount ($\mu\text{g/mL}$)	Recovery (%)	RSD (%)
Daidzein	Low	20.15	21.58	107.09	0.13
	Medium	35.26	38.15	108.16	2.40
	High	50.37	56.16	111.50	0.08
Interday					
Compounds	Level	Added amount ($\mu\text{g/mL}$)	Found amount ($\mu\text{g/mL}$)	Recovery (%)	RSD (%)
Daidzein	Low	20.15	19.89	98.73	1.06
	Medium	35.26	39.05	110.40	0.69
	High	50.37	56.07	111.32	0.36

Sensitivity

The LOD and LOQ were determined using standard deviation approach according to Miller & Miller¹². LOD and LOQ values for daidzein were 0.16 and 0.47 µg/mL respectively.

Content Determination of Daidzein

The optimized and validated RP-HPLC method was applied to determine the daidzein content in soy sauce samples. From six replications of determination, it was found that the content of daidzein in soy sauce samples was 0.0014% (w/w) or 0.014 mg/g dry weight with the RSD value of 0.90. According to the results, it was found that the RSD value met the requirements of <5.30%²¹. The daidzein levels that tend to be obtained are lower than the results of previous study²². Witold et al. stated that daidzein content were 0.105 – 0.850 mg/g from dry weight. This variation is possibly occurred due to the variations in the types of soybeans, soy sauce processing, and fermentation.

Conclusions

In this study, an RP-HPLC method was successfully optimized by employing the RSM model to evaluate the content of daidzein in soy sauce samples. The optimized condition was applied in the analytical method validation stage. The developed RP-HPLC method met the requirements for validation variables including selectivity, linearity and range, sensitivity, accuracy, and precision. It was also possible to apply the RP-HPLC method for determining the content of daidzein in soy sauce samples.

Acknowledgements

We thank to Institute of Research and Community Services, Sanata Dharma University for financially fund with Young Lecturer Research Grant (“Penelitian Dosen Muda”) of 2023, with contract number of No. 013/Penel/LPPM-USD/II/2023. We also thank Universitas Gadjah Mada for supporting the purchase of reference standards used in this study.

REFERENCES

1. M. Messina, "Soy and health update: Evaluation of the clinical and epidemiologic literature", *Nutrients*, 8(12), 1–42 (2016).
2. S. Hassan, "Soybean, Nutrition and Health", in *Soybean - Bio-Active Compounds InTech*, , 453–473 (2013).
3. I-S. Kim, "Current Perspectives on the Beneficial Effects of Soybean Isoflavones and Their Metabolites for Humans", *Antioxidants (Basel)*, 10(7),1064 (2021).
4. Z. Huang, M. Zhao, C. Cui, M. Huang, L. Lin and Y. Feng, "A new sight on soy isoflavones during the whole soy sauce fermentation process by UPLC-MS/MS", *LWT*, 152, 112249 (2021).
5. M. M. Alshehri, J. Sharifi-Rad, J. Herrera-Bravo, E. L. Jara, L. A. Salazar, D. Kregiel, Y. Uprety, M. Akram, M. Iqbal, M. Martorell, M. Torrens-Mas, D. G. Pons, S. D. Daştan, N. Cruz-Martins, F. A. Ozdemir, M. Kumar and W. C. Cho, "Therapeutic Potential of Isoflavones with an Emphasis on Daidzein", *Oxid Med Cell Longev*, 2021, 1–15 (2021).
6. L. Y. Yoshiara, T. B. Madeira, F. Delaroza, J. B. Da Silva and E. I. Ida, "Optimization of soy isoflavone extraction with different solvents using the simplex-centroid mixture design", *Int J Food Sci Nutr*, 63(8), 978–986 (2012).
7. Kemenkes RI, "Farmakope Indonesia Edisi VI", Kementerian Kesehatan Republik Indonesia, Jakarta, (2020).
8. F. D. O. Riswanto, A. Rohman, S. Pramono and S. Martono, "Employing an R software package rsm for optimizing of genistein, daidzein, and glycitein separation and its application for soy milk analysis by HPLC method", *Indones J Chem*,20(5), 1184–1198 (2020).
9. J. Lawson, "Design and Analysis of Experiments with R", Chapman and Hall/CRC, (2014).
10. S. H. Yuliani, M. R. Gani, E. P. Istyastono and F. D. O. Riswanto, "Optimization of genistein and daidzein extraction from a tempeh- fermented product of soybean", *J Pharm Pharmacogn Res*, 6(4), 231–241 (2018).
11. F. D. O. Riswanto, A. Rohman, S. Pramono and S. Martono, "Analytical Method Validation and Determination of Genistein, Daidzein, and Glycitein in

- Soybean Milk by RP-HPLC Method", *Int J Pharm Res*, 13, 2590–2595 (2021).
12. J. N. Miller, J. C. Miller and R. D. Miller, "Statistics and Chemometrics for Analytical Chemistry" Pearson Education Limited, Harlow, Seventh., (2018).
 13. T. Farihah, "Penentuan Pola Kelelahan Fisik Pada Perokok Aktif Dengan Menggunakan Metode Response Surface Methodology", *J@ti Undip J Tek Ind*, 11(2), 107 (2016).
 14. E. Sulistyowati, S. Martono, S. Riyanto and E. Lukitaningsing, "Analysis of Daidzein and Genistein in Soybean (Glycine max L. Merril) Anjasmoro, Argomulyo and Dena 2 Varieties Using HPLC Method (Original title in Indonesian: Analisis Daidzein dan Genistein pada Kedelai (Glycine max L. Merril) Varietas Anjasmoro", *Argom Media Farm Indones*, 13, 1299–1304 (2018).
 15. T. R. Handoyo, G. A. Purnomo, C. D. Maryanto and M. R. Gani, "Validasi Dan Penetapan Kadar Senyawa Rutin Pada Ekstrak Etanol Daun Binahong (Anredera cordifolia (Ten.) Steenis) Dengan Metode KCKT", *Fitofarmaka J Ilm Farm*, 12, 1–13 (2022).
 16. Merck, "A Practical Guide to High Performance Liquid Chromatograph" Merck, Darmstadt, (2021).
 17. R. Dwiastuti, D. C. A. Putri, M. Hariono and F. D. O. Riswanto, "Multiple Response Optimization of a HPLC Method for Analyzing Resorcinol and 4-n-Butyl Resorcinol in Lipid Nanoparticles", *Indones J Chem*, 21(2), 502 (2021).
 18. S. Winarni, B. Handoko and Y. Krista, "Desirability Function with Principal Component Analysis for Multi-response Optimization", in *Desirability Function with Principal Component Analysis for Multi-Response Optimization Universitas Padjajaran, Bandung*, 2, 171–178 (2016).
 19. J. . Snyder, L., Kirkland and J.J., Dolan, "Introduction to Modern Liquid Chromatography", Third Edition, 22 (2010).
 20. C. K. Sahoo, M. Sudhakar, N. K. Sahoo, S. Ram, M. Rao and U. P. Panigrahy, "Validation of Analytical Methods: A Review - includes specificity and selectivity definitions", *Int J Chromatogr Sep Tech*, 2018, 112 (2018).
 21. AOAC, AOAC Official Methods of Analysis. Assoc. Off. Agric. Chem. Washington, D.C., doi: 10.3390/s150304766 (2016).
 22. W. M. Mazur, J. A. Duke, K. Wähälä, S. Rasku and H. Adlercreutz, "Isoflavonoids and Lignans in Legumes: Nutritional and Health Aspects in Humans", 11The method development and synthesis of the standards and deuterium-labelled compounds was supported by National Institutes of Health Grants No. 1 R01 CA56289-01 and No. 2 R0, *J Nutr Biochem*, 9, 193–200 (1998).



نشرة العلوم الصيدلانية جامعة أسيوط



التحسين القائم على منهجية السطح الاستجابي لطريقة كروماتوغرافيا السائل عالية الأداء ذات الطور العكسي ، التحقق من صحة الطريقة التحليلية، وتحديد محتوى الدايزين في صلصة الصويا

فلورنتينوس ديكا أوكتا ريسوانتو^{١*} - جوفان تري ويبوو^١ - إيميرينتيانا ريكا إستري وولانجاري^١ -
كيتيرين سادنا مارتياس^١ - مايكل راهارجا جاني^١ - سوديبو مارتونو^١

^١ قسم التحليل الصيدلي والكيمياء الطبية، كلية الصيدلة، جامعة سانانا دارما، يوجياكارتا ٥٥٢٨٢، إندونيسيا

^٢ المجموعة البحثية لتصميم الأدوية بمساعدة الكمبيوتر واكتشاف المنتجات الطبيعية النشطة بيولوجياً، كلية

الصيدلة، جامعة سانانا دارما، يوجياكارتا ٥٥٢٨٢، إندونيسيا

يُعد فول الصويا من الأطعمة التي تستهلك بشكل واسع في إندونيسيا ويُعرف بفوائده الصحية للإنسان. ومن بين منتجات فول الصويا هو صلصة الصويا. وتشير التقارير إلى أن فول الصويا ومنتجاته تحتوي على الدايزين ، وهو أحد مركبات الأيزوفلافون التي توفر أنشطة بيولوجية مفيدة للبشر. لذلك، من المهم تطوير طريقة تحليلية مناسبة لتحليل محتوى الـ الدايزين في صلصة الصويا. تم استخدام تصميم بوكس بهنكن، وهي منهجية شائعة للأسطح الاستجابي، لتحسين المتغيرات المستقلة مثل تكوين الطور المتحرك من الميثانول والأسيتونيتريل وظروف معدل التدفق. تم تقييم التحقق من صحة الطريقة التحليلية بما في ذلك الخطية والنطاق، الانتقائية، الدقة، والتكرارية في هذه الدراسة. كانت الشروط المثلى لتحليل الـ الدايزين هي الطور المتحرك من الميثانول:الماء:الأسيتونيتريل (٥:٣٥:٦٠ v/v/v)، ومعدل تدفق ١.٠ مل/دقيقة، وطول موجة الكشف عند ٢٥١ نانومتر. حققت هذه الشروط متطلبات اختبار ملائمة النظام مع تفاوت منخفض لعوامل التموج، الفصل، وقت الاحتفاظ، عدد الألواح النظرية، ومساحة تحت المنحنى. تم التحقق من صحة الطريقة التحليلية بنجاح لعدة معايير بما في ذلك الخطية والنطاق، الانتقائية، الدقة، والتكرارية. يمكن تحسين طريقة كروماتوغرافيا السائل عالية الأداء ذات الطور العكسي لتحليل الدايزين في صلصة الصويا باستخدام تصميم بوكس بهنكن وحققت هذه الطريقة معايير قبول التحقق من صحة الطريقة التحليلية من حيث الخطية والنطاق، الانتقائية، الدقة، والتكرارية. وفقاً لنتائج التحليل الكمي، وُجد أن محتوى الدايزين في عينة صلصة الصويا بلغ ٠.٠١٤ ملغم/غرام.