CURRENT STATUS OF ANALYTICAL TECHNIQUES USED FOR THE ANALSIS OF BERBERINE AND ITS SALTS

Kanchan Devi and Surajpal Verma*

School of pharmaceutical sciences, Lovely professional university, Phagwara-144411, Punjab, India

Abstract

Berberine is a natural alkaloid obtained from different species of *Berberis* like *Berberis aristata, vulgaris, aquifolum* and so on having family berbediaceae. It is also called barberry. It has multispectral uses but its less oral bioavailability (<1%) limits its use. The water solubility of berberine is 0.000354mg/ml that can be increased by addition of salts like hydrochloride, fumarate, citrate etc. The melting point of Berberine is 193-196 ° C and molecular weight is 372.5g/mol. It is used in the treatment of various diseases like diabetes, cancer, antimicrobial, anti-inflammatory, antiprotozoal and so on. Berberine is available in different dosage forms like tablets, beads, topicals gels and hydrogels pellets and various vesicular delivery systems. The log P, tPSA, %HSA of berberine was found to be −1.5, 40.9 A &25.98%, that means berberine is hydrophilic in nature. Berberine is mainly checked by HPLC, IR/HPLC-UV, HPLC and micro dialysis, CE/ESI-MS, Surface enhanced Raman scattering (SERS), UPLC/MS-MS, HPLC/ESI-MS, HPTLC, HPLC-UV etc. It is mostly analyzed by HPLC and Mass spectrometer. In current we are going to discuss about the Berberine, pharmacokinetic properties, physicochemical properties, uses of berberine, different available dosage forms and the analytical methods used to analyze berberine and its salts.

Keywords

Berberine, Antimicrobial, Hydrogels pellets, Surface enhanced Raman scattering, Mass spectrometer

INTRODUCTION

Berberine is a natural isoquinoline quaternary alkaloid derived from a number of species including Coptis *chinensis* (Coptis or Goldthread), *Hydrastis canadensis* (goldenseal), *Berberis aquifolium* (Oregon grape), *Berberis aristata* (Tree Turmeric), *Berberis vulgaris* (Barberry), & *Arcangelisia flava* family berbediacea(1). It has very long traditional history and used both in Chinese as well as Ayurvedic medicines. There are many chemical constituents present in berberis that are mainly present in roots, stem and rhizomes of the plant. It is used as hypoglycemic,

anti-inflammatory, against tumour, anti-diarrheal, anti-protozoal, antiviral, and antibacterial activities, anti-microbial etc.(2). Thus, the drug has emerged as a medicinal agent with multispectral activities(3). Berberine is not used much for research because of the poor bioavailability problems (4).Berberine is now available with many salts that increases the solubility of the drug like hydrochloride, fumarate, malate, succinate, citrate etc.(5).

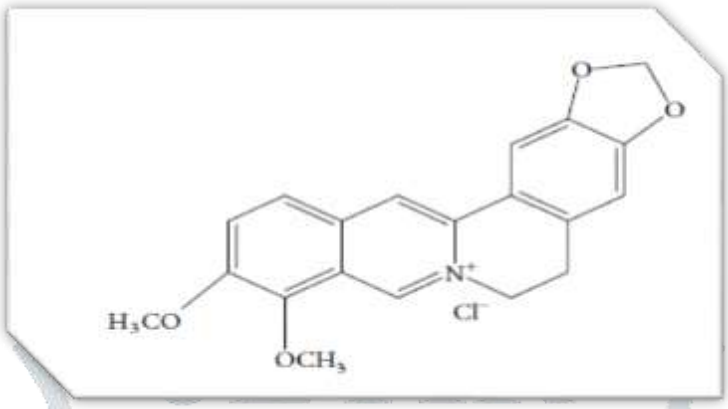


Figure 1: Structure of Berberine

DRUG PROFILE

Drug: Berberine

Chemical name: 5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]

Molecular formula: $C_{20}H_{19}NO_5$

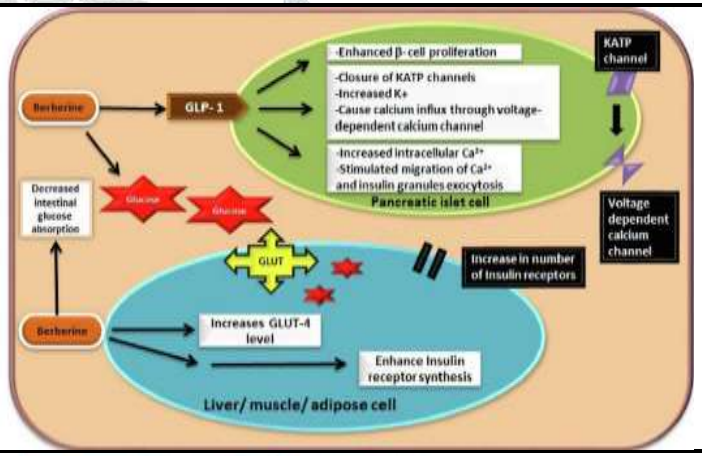
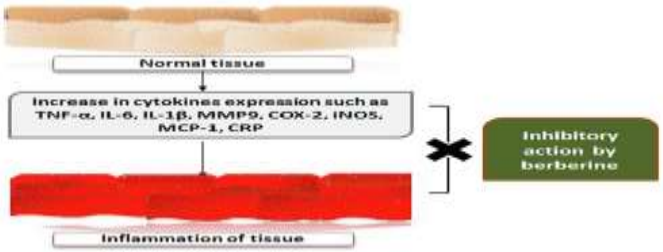
Table 1: Drug profile of Berberine(5),(6)**.**

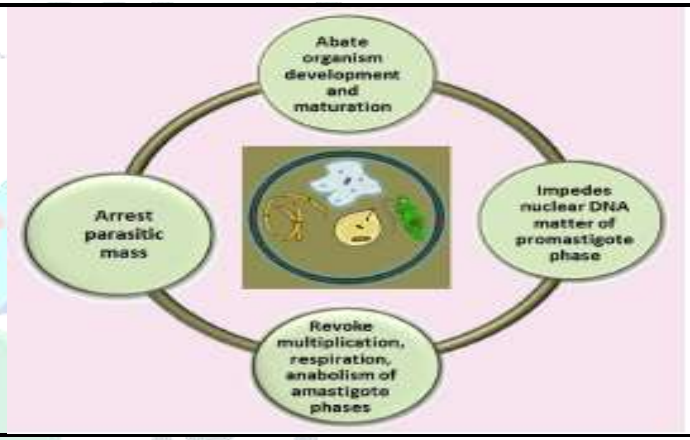
Synonym	Barberry
Color	Intense yellow
Melting point	193-196 $^{\circ}$C
Solubility	Easily soluble in water, ethanol & sparingly soluble in CH_3O
Half life	11.79± 3.51
Category	Alkaloid
Molecular weight	336.5g/mol

Absolute bioavailability	0.68%
Clearance	60.72±6.00
Taste	Bitter
Odour	Odorless

Uses of berberine-

Table 2: Uses of berberine

S.no	Effect	Structure	Reference
Anti-diabetic:	• Berberine show similar action as metformin i.e., improve insulin sensitivity in high-fat diet rats and also act as an insulin stimulating agent in cell culture studies. • By activating AMPK.		(7),(8)
Anti-inflammatory	• Through AMPK pathway, it stops the formation of COX-2, TNF- α, Inter leukins-6 etc.		(9)

Anti- cancer	• Berberine retards the DNA repair, causes cell death on dose up to 200 μM		(3)
Anti-protozoal	• Retardation of Giardia intestinalis, Trichomonas vaginalis.		(3) (8)

Pharmacokinetic profile of berberine

Table 3: Pharmacokinetic profile of berberine

S.no	Pharmacokinetic profile	Reference
1.	**Absorption:** Bioavailability 0.68% (Mode- Femoral vein) 0.37% of bioavailability (Intragastric administration)	(10)(11)(12)
2.	**Distribution:** liver > kidneys > muscle > lungs > brain > heart > pancreas > fats.	(13)(14)(15)
3.	**Biotransformation:** Intestine & liver are involved in the biotransformation of berberine. Mainly Cytochrome P450 is responsible for metabolism.	(16)(17)(18)
4.	**Excretion:** 22.83% in bile, 0.0941% in urine and 23.65% in waste product.	(19)(8)(20)

PHYSICOCHEMICAL PROPERTIES

Table 4: Pharmacochemical properties

S.no	Parameter	Value	Reference
1.	pKa	15.7	(21)
2.	Solubility	5.27 ± 0.29mM	(22)
3.	Log P	-1.4	(23)
4.	tPSA	40.9	(24)
5.	%HSA	25.97%	(8)

DOSAGE FORMS

Berberine is available in different dosage forms like tablets, pellets, beads, solid dispersions, topical gels and hydrogels, SMEDDS(25), micelles(26) etc.

Table 5: Dosage forms of berberine

Dosage form	Formulations	Reference
Tablet	• Formulation having Eudragit L30D-55/S100 as a coating material. • Berberine floating delivery system (FDS)	(27) (28)
Pellets	• By using chitosan & alginate, berberine pellets were prepared. • By using Eudragit (RL PO& PS PO) pellets were prepared	(9) (4)
Bead	• Beads of Ca alginate were prepared of berberine.	(2)
Topical gels and hydrogels	• CMC and hyaluronic acid were used to prepare gel of berberine	(29) (30)
Solid dispersions	• To increase absorption, Na caprate was used to prepare solid dispersion of berberine. • Solid dispersion was prepared using HPC of berberine. • By using Eudragit solid dispersion of berberine was prepared.	(31) (32) (33) (34)

0

ANALYTICAL TECHNIQUES

Table 6: Analytical techniques

S.no	Instrument	Flow rate (ml/min)	Injection volume(µl)	Mobile phase	Temperature of column (° C)	Column type	Wavelength	Salts of berberine	Reference
1.	**HPLC-UV**	1.0ml/min	10µl	Potassium hydrogen phosphate and acetonitrile	40±1°C	Reverse phase	346nm	Berberine chloride	(29)
2.	**HPLC**	1.0ml/min	20µl	ACN: Potassium dihydrogen phosphate: Sodium dodecyl sulphate (50:50:0.06)	35°C	C_{18}	345nm	Berberine hydrochloride, fumarate, malate, succinate and citrate	(5)
3.	**HPTLC**	-	-	Ethyl acetate, formic acid, Toluene, CH_3OH (9:3:9:1)	-	-	350nm	Berberine	(30)
4.	**HPLC-ESI-MS A: HPLC Conditions-**	0.2ml/min	10µl	**Mobile phase:** a- (0.09% formic acid and 2 mmol/l $C_2H_7NO_2$ b- ACN	40°C	C_{18}	-	Berberine	(31)

	B: Mass Conditions	**Nebulizer gas-** Liquid nitrogen **Drying gas-** curtain gas	**Gas flow rate-** 4.01ml/min	**Voltage:** Probe:4.5kV CDL: -10V Detector:1.65kV	200-250 ˚ C	-	-	Berberine	
5.	**UPLC/MS-MS A: UPLC conditions**	-	2.0µl	**Mobile phase**: (A) acetonitrile (B) water	40 ˚C	C_{18}	-	Berberine hydrochloride	(32)
	B: Mass conditions-	**Gas-** curtain gas	**Collision energy:** 40eV	**Ion spray voltage:** 4500 V	**Declustering potential:** 65s	**Temp:** 400˚ C	**Dwell time:** 100ms	**Detector mode:** MRM mode	
6.	**IR/HPLC-UV**	0.7ml/min	10µl	Tetrabutylammonium chloride and acetonitrile	Room temperature	Therom ODS Hypersil column	346nm	Berberine	(33)
7.	**LC/ESI-MS A: HPLC conditions:**	1.0ml/min	-	**Mobile phase:** a: ACN b: (10mm C_2 H_7 NO_2 solution having 0.1% formic acid)	25 ˚C	C_{18}	-	Berberine hydrochloride	(34)

	B: Mass Condition:	**Drying gas:** (N2)	**Gas flow:** 12 ml/min	**Nebulizing pressure:** 40 pascals	**Gas drying temperature** 350 ˚C	**Voltage** **Capillary:** 3 kV	**Fragmentor voltage**: 160V	Berberine	
8.	**CE/ESI-MS A: Capillary Conditions:**	**CE columns**: fused-silica capillaries	**Length of capillary:** 85cm	-	-	-	-	Berberine	(35)
	B: Mass conditions:	**Mode**- positive ion mode	**Data collection**: SIM (Selected ion monitoring)	**Potential difference:** 20kV	-	-	-	Berberine	
9.	**Narrow bore HPLC with ion pair extraction**	**Ion pair reagent:** Sodium dodecyl sulfate	**Conc. Of reagent:** 17–50 mM	**Mobile phase:** ACN in 0.05 M phosphate buffer with10 mM $C_8H_{17}NaO_3S$	-	C_{18}	-	Berberine	(36)

Conclusion- Berberine is a potential molecule that is used to treat variety of diseases and has clinical applications. Hypoglycemic, anti- cancer, anti- protozoal and anti- inflammatory action of berberine is discussed along with physicochemical properties and pharmacokinetic profile. Berberine is available in different dosage forms like tablets, pellets, beads, solid dispersions, topical gels and hydrogels, SMEDDS, micelles etc. If we talk about the analytical methods, berberine and its salts are analyzed by HPLC, IR/HPLC-UV, HPLC and micro dialysis, CE/ESI-MS, Surface enhanced Raman scattering (SERS), UPLC/MS-MS, HPLC/ESI-MS, HPTLC, HPLC-UV etc. It is mostly analyzed by HPLC and Mass spectrometer. Berberine will be analyzed by more sensitive and hyphenated techniques in future.

REFERENCES:

1. Pang B, Zhao L-H, Zhou Q, Zhao T-Y, Wang H, Gu C-J, et al. Application of Berberine on Treating Type 2 Diabetes Mellitus. Int J Endocrinol [Internet]. 2015;2015:1–12. Available from: http://www.hindawi.com/journals/ije/2015/905749/
2. Zhang Z-H, Sun Y-S, Pang H, Munyendo WLL, Lv H-X, Zhu S-L. Preparation and Evaluation of Berberine Alginate Beads for Stomach-Specific Delivery. Molecules [Internet]. 2011 Dec 14;16(12):10347–56. Available from: http://www.mdpi.com/1420-3049/16/12/10347
3. Vuddanda PR, Chakraborty S, Singh S. Berberine: a potential phytochemical with multispectrum therapeutic activities. Expert Opin Investig Drugs [Internet]. 2010 Oct 13;19(10):1297–307. Available from: http://www.tandfonline.com/doi/full/10.1517/13543784.2010.517745
4. Gao Y, Jin X, Sun Y, Xu FF, Zhang M. Production and investigation of sustained berberine pellet drug release system. Adv Powder Technol [Internet]. 2017 Mar;29(3):682–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0921883117305058
5. Cui H-X, Hu Y-N, Li J-W, Yuan K, Guo Y. Preparation and Evaluation of Antidiabetic Agents of Berberine Organic Acid Salts for Enhancing the Bioavailability. Molecules [Internet]. 2019 Dec 28;24(1):103. Available from: http://www.mdpi.com/1420-3049/24/1/103
6. Mirhadi E, Rezaee M, Malaekeh-Nikouei B. Nano strategies for berberine delivery, a natural alkaloid of Berberis. Biomed Pharmacother [Internet]. 2018 Aug;104:465–73. Available from: https://doi.org/10.1016/j.biopha.2018.05.067
7. Li Z, Geng Y-N, Jiang J-D, Kong W-J. Antioxidant and Anti-Inflammatory Activities of Berberine in the Treatment of Diabetes Mellitus. Evidence-Based Complement Altern Med [Internet]. 2014;2014:1–12. Available from: http://www.hindawi.com/journals/ecam/2014/289264/
8. Raju M, Kulkarni YA, Wairkar S. Therapeutic potential and recent delivery systems of berberine: A wonder molecule. J Funct Foods [Internet]. 2019 Oct;61(June):103517. Available from: https://doi.org/10.1016/j.jff.2019.103517
9. Zhang X, Zhang X, Wang C, Li Y, Dong L, Cui L, et al. Neuroprotection of early and short-time applying berberine in the acute phase of cerebral ischemia: Up-regulated pAkt, pGSK

and pCREB, down-regulated NF-κB expression, ameliorated BBB permeability. Brain Res [Internet]. 2012 Jun;1459:61–70. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006899312006221

10. Chen W, Miao Y-Q, Fan D-J, Yang S-S, Lin X, Meng L-K, et al. Bioavailability Study of Berberine and the Enhancing Effects of TPGS on Intestinal Absorption in Rats. AAPS PharmSciTech [Internet]. 2011 Jun 3;12(2):705–11. Available from: http://link.springer.com/10.1208/s12249-011-9632-z
11. Liu Y-T, Hao H-P, Xie H-G, Lai L, Wang Q, Liu C-X, et al. Extensive Intestinal First-Pass Elimination and Predominant Hepatic Distribution of Berberine Explain Its Low Plasma Levels in Rats. Drug Metab Dispos [Internet]. 2010 Oct;38(10):1779–84. Available from: http://dmd.aspetjournals.org/lookup/doi/10.1124/dmd.110.033936
12. Hano M, Tomášová L, Šereš M, Pavlíková L, Breier A, Sulová Z. Interplay between P-Glycoprotein Expression and Resistance to Endoplasmic Reticulum Stressors. Molecules [Internet]. 2018 Feb 6;23(2):337. Available from: http://www.mdpi.com/1420-3049/23/2/337
13. Tan X-S, Ma J-Y, Feng R, Ma C, Chen W-J, Sun Y-P, et al. Tissue Distribution of Berberine and Its Metabolites after Oral Administration in Rats. Deli MA, editor. PLoS One [Internet]. 2013 Oct 31;8(10):e77969. Available from: http://dx.plos.org/10.1371/journal.pone.0077969
14. Wang X, Xing D, Wang W, Su H, Tao J, Du L. Pharmacokinetics of Berberine in Rat Thalamus After Intravenous Administration of Coptidis Rhizoma Extract. Am J Chin Med [Internet]. 2005 Jan 5;33(06):935–43. Available from: https://www.worldscientific.com/doi/abs/10.1142/S0192415X05003557
15. Li Z, Wei Y, Chu T. Radioiodination, biodistribution and pharmacokinetics of berberine in mice. J Radioanal Nucl Chem [Internet]. 2005 Aug;265(3):355–9. Available from: http://link.springer.com/10.1007/s10967-005-0832-4
16. Zuo F, Nakamura N, Akao T, Hattori M. Pharmacokinetics of Berberine and Its Main Metabolites in Conventional and Pseudo Germ-Free Rats Determined by Liquid Chromatography/Ion Trap Mass Spectrometry. Drug Metab Dispos [Internet]. 2006 Dec;34(12):2064–72. Available from: http://dmd.aspetjournals.org/lookup/doi/10.1124/dmd.106.011361

17. Li Y, Ren G, Wang Y-X, Kong W-J, Yang P, Wang Y-M, et al. Bioactivities of berberine metabolites after transformation through CYP450 isoenzymes. J Transl Med [Internet]. 2011;9(1):62. Available from: http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-9-62
18. Liu J, He C, Zhou K, Wang J, Kang JX. Coptis extracts enhance the anticancer effect of estrogen receptor antagonists on human breast cancer cells. Biochem Biophys Res Commun [Internet]. 2009 Jan;378(2):174–8. Available from: http://dx.doi.org/10.1016/j.bbrc.2008.10.169
19. Ma J, Feng R, Tan X, Ma C, Shou J, Fu J, et al. Excretion of Berberine and Its Metabolites in Oral Administration in Rats. J Pharm Sci [Internet]. 2013 Nov;102(11):4181–92. Available from: http://dx.doi.org/10.1002/jps.23718
20. Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: An update. Eur J Pharmacol [Internet]. 2015 Aug;761:288–97. Available from: http://dx.doi.org/10.1016/j.ejphar.2015.05.068
21. Roja G, Bhangale AS, Juvekar AR, Eapen S, D'Souza SF. Enhanced Production of the Polysaccharide Arabinogalactan Using Immobilized Cultures of Tinospora cordifolia by Elicitation and In Situ Adsorption. Biotechnol Prog [Internet]. 2005 Dec 2;21(6):1688–91. Available from: http://doi.wiley.com/10.1021/bp050188w
22. Spinozzi S, Colliva C, Camborata C, Roberti M, Ianni C, Neri F, et al. Berberine and Its Metabolites: Relationship between Physicochemical Properties and Plasma Levels after Administration to Human Subjects. J Nat Prod [Internet]. 2014 Apr 25;77(4):766–72. Available from: https://pubs.acs.org/doi/10.1021/np400607k
23. Battu SK, Repka MA, Maddineni S, Chittiboyina AG, Avery MA, Majumdar S. Physicochemical Characterization of Berberine Chloride: A Perspective in the Development of a Solution Dosage Form for Oral Delivery. AAPS PharmSciTech [Internet]. 2010 Sep 15;11(3):1466–75. Available from: http://link.springer.com/10.1208/s12249-010-9520-y
24. Zhang Y, Cui Y-L, Gao L-N, Jiang H-L. Effects of β-cyclodextrin on the intestinal absorption of berberine hydrochloride, a P-glycoprotein substrate. Int J Biol Macromol [Internet]. 2013 Aug;59:363–71. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2013.04.074

25. Zhu J-X, Tang D, Feng L, Zheng Z-G, Wang R-S, Wu A-G, et al. Development of self-microemulsifying drug delivery system for oral bioavailability enhancement of berberine hydrochloride. Drug Dev Ind Pharm [Internet]. 2013 Mar 8;39(3):499–506. Available from: http://www.tandfonline.com/doi/full/10.3109/03639045.2012.683875
26. Wang T, Wang N, Song H, Xi X, Wang J, Hao A, et al. Preparation of an anhydrous reverse micelle delivery system to enhance oral bioavailability and anti-diabetic efficacy of berberine. Eur J Pharm Sci [Internet]. 2011 Sep;44:127–35. Available from: http://dx.doi.org/10.1016/j.ejps.2011.06.015
27. Ji J, He X, Yang X-L, Du W-J, Cui C-L, Wang L, et al. The In vitro/vivo Evaluation of Prepared Gastric Floating Tablets of Berberine Hydrochloride. AAPS PharmSciTech [Internet]. 2017 Aug 29;18(6):2149–56. Available from: http://link.springer.com/10.1208/s12249-016-0696-7
28. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating drug delivery systems: A review. AAPS PharmSciTech [Internet]. 2005 Sep;6(3):E372–90. Available from: http://link.springer.com/10.1208/pt060347
29. Singh R, Katiyar C, Pasrija A. Validated HPLC-UV method for the determination of berberine in raw herb Daruharidra (Berberis aristata DC), its extract, and in commercially marketed ayurvedic dosage forms. Int J Ayurveda Res [Internet]. 2010;1(4):243. Available from: http://www.ijaronline.com/text.asp?2010/1/4/243/76789
30. Ghosh VK, Nagore DH, Patil MJ, Prakash A. Development and Validation of a Method for Densitometric Analysis of Berberine in Herbal Extract and Polyherbal Formulation. Med Princ Pract [Internet]. 2010;19(6):473–8. Available from: https://www.karger.com/Article/FullText/320307
31. Lu T, Liang Y, Song J, Xie L, Wang GJ, Liu XD. Simultaneous determination of berberine and palmatine in rat plasma by HPLC–ESI-MS after oral administration of traditional Chinese medicinal preparation Huang-Lian-Jie-Du decoction and the pharmacokinetic application of the method. J Pharm Biomed Anal [Internet]. 2006 Mar;40(5):1218–24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S073170850500645X
32. Li G, Yang F, Liu M, Su X, Zhao M, Zhao L. Development and application of a UPLC-MS/MS method for simultaneous determination of fenofibric acid and berberine in rat plasma: application to the drug-drug pharmacokinetic interaction study of fenofibrate

combined with berberine after oral administrati. Biomed Chromatogr [Internet]. 2016 Jul;30(7):1075–82. Available from: http://doi.wiley.com/10.1002/bmc.3652

33. Chan C-O, Chu C-C, Mok DK-W, Chau F-T. Analysis of berberine and total alkaloid content in Cortex Phellodendri by near infrared spectroscopy (NIRS) compared with high-performance liquid chromatography coupled with ultra-visible spectrometric detection. Anal Chim Acta [Internet]. 2007 Jun;592(2):121–31. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0003267007007416
34. Hua W, Ding L, Chen Y, Gong B, He J, Xu G. Determination of berberine in human plasma by liquid chromatography–electrospray ionization–mass spectrometry. J Pharm Biomed Anal [Internet]. 2007 Aug;44(4):931–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0731708507001914
35. Chen YR, Wen KC, Her GR. Analysis of coptisine, berberine and palmatine in adulterated Chinese medicine by capillary electrophoresis–electrospray ion trap mass spectrometry. J Chromatogr A [Internet]. 2000 Jan;866:273–80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021967399011152
36. Lee HS, Eom YE, Eom DO. Narrowbore high performance liquid chromatography of berberine and palmatine in crude drugs and pharmaceuticals with ion-pair extraction using cobalt thiocyanate reagent. J Pharm Biomed Anal. 1999;21:59–63.