Development and validation of RP- HPLC method with UV detection to determine and quantify dimenhydrinate in human plasma

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Abstract: A simple, sensitive and rigorous method for estimation of dimenhydrinate in human plasma was searched and its validation was carried out. LLE (Liquid-Liquid extraction) of analyte with mixture of Hexane and ethyl acetate (1:1 v/v) was carried out for the preparation of Plasma Samples, Chromatographic elution of dimenhydrinate was conducted in human plasma and mobile phase with C-18 bonda Pack column (10µm; 250 × 4.6), using a mobile phase consisting a solution of ammonium bicarbonate in water and methanol at a flow rate of 0.5ml/minute with UV detection at 229 nm. The resolution of dimenhydrinate was well performed from plasma components. This method was validated and exhibited linearity with concentration range of 6 to 380ng/ml of dimenhydrinate in plasma. The Intra day precision was 89.2 to 96.89% and Inter day precision was 88.6% to 93.26%, the average recovery of dimenhydrinate was 97.02%. The efficacy of extraction was proved by above mentioned results. 2ng/ml and 6ng/ml, were appraised as the LOD and LOQ of dimenhydrinate, stability studies disclosed that dimenhydrinate exhibited stability in Plasma after Freeze & thaw cycles and upon -20°C storage, the method was developed well.

Keywords: Dimenhydrinate, human plasma, HPLC, limit of detection, analyte.

INTRODUCTION

Dimenhydrinate (DMH) (fig. 1) is salt of 8-chlorotheophylline. Chemical composition of the salt is 8-chloro-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione compound with 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine (1:1) (Moffat *et al.,* 2004). It is an antiemetic drug. Dimenhydrinate uses medically to treat nausea, vomiting and vertigo due to Meniere's disease and other vestibular disease (Sweetman, 2009). HPLC is suggested by the United States Phaemacopeia for the assay of dimenhydrinate (United States Pharmacopeia, 2011). The quantification of dimenhydrinate in biological fluids was mentioned by some researchers. This data shows different analytical procedures used for appraisement for example Spectrophotometry (Karr *et al.*, 1981; Mitic *et al.*, 2012). UV spectroscopic multisensory (Canada *et al*., 1999) adsorption strip voltammetry, (Shubeitah *et al.*, 1999), electrophoresis (Ong *et al*, 1991) and HPLC-tandem mass spectrometry (LC-MS-MS) (Tavares *et al.*, 2007), HPLC fluorescence detection (Ozkan *et al.*, 2013; Barbas *et al.*, 2000) and HPLC with UV detection (Doge and Egar, 2007) have been put forwarded . An HPTLC method had been also published (Digregorio and Sherma, 2000; EL -Kafraway and Belal, 2016).

The process for the recognition and quantification of Dimenhydrinate impurities in finished products was evolved using forced degradation studies (Girish *et al.*, 2016). Synchronal identification of cinnarizine (CIN) and dimenhydrinate (DIM), was evolved and validated by using thin layer chromatography (TLC) spectrodensito metric evaluation. Another method is high-performance liquid chromatography. The methods were conveniently applied to analyze raw materials and pharmaceutical dosage forms (Lamie *et al.*, 2016). Another method was produced for contemporaneous identification of dimenhydrinate and cinnarizine, using spectrophometric analysis (Lamie *et al.*, 2015). Dimenhydrinate loaded buccal bio adhesive films were produced and optimized for prolonged release (Pekoz *et al.*, 2015).

Fast dissolving buccal adhesive films were produced and assessed successfully (Andrea *et al.*, 2016). Titremetric analysis for dimenhydinate in raw powders and finished forms is recommends by Monograph of British Pharmacopeia (British Pharmacopeia, 2010).

Several methods for analysis have been enquired and reported for quantification of dimenhydrinate in plasma fluids, for example electro spray tandam mass spectrometry. High pressure liquid chromatography (HPLC) with ultra violet (UV) detection in conjunction with gas chromatography (GC) although they are impressible but lack simplicity and modesty.

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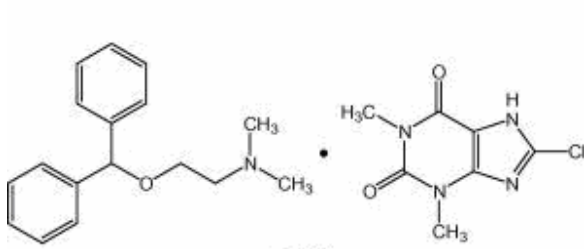
The present method illustrates a specific and rapid (HPLC) method to estimate dimenhydrinate in human plasma, the method based on extraction of dimenhydrinate in human plasma using Hexane ethyle acetate mixture (Tavares *et al.*, 2007) and USP method for assay was adapted (United States Pharmacopeia, 2011). The limits of detection (LOD) and limits of quantification (LOQ) are better as compared to other analytical methods on HPLC for quantification and assay of dimenhydrinate.



DMH

MATERIALS AND METHODS

Chemicals & reagents

Dimenhydrinate was gifted from Searle Pakistan private limited. Hexane, ethyl-acetate, Ammonium Bicarbonate all chemicals were picked from best analytical grade (Merck). De ionized water was utilized for testing.

Composition of mobile phase

Ammonium bicarbonate solution A & B, (Solution A-Dissolve 0.8g of ammonium bicarbonate in 800ml of water. Add 200ml of methanol filtered and degassed. Solution B-Dissolve 0.8 gm of ammonium bicarbonate in 150 ml of water. Add 850ml of methanol, filtered and degassed. Flow rate was 0.ml/min, injection volume was 10µl and wave length was 229nm.

Preparation of standards and quality control samples

Ammonium bicarbonate solution -0.016g/ml of ammonium bicarbonate in water. Diluent In 0.02gm/ml solution of ammonium bicarbonate in water, 50ml of methanol was added and mixed.

Standard preparation- In 50 mg of dimenhydrinate, 5ml of ammonium bicarbonate solution and 20ml of methanol was added and mixed, to 1 ml of this 9ml of diluent was added and mixed. Working standard solution 5ml of stock solution was taken and volume was made up to100ml with diluent.

Seven working Standards Containing 6,11.25,22.5,45,90, 180 and 360ng/ml of dimenhydrinate were prepared with appropriate volumes of ammonium bicarbonate solution, all stock solution were refrigerated (2-8^{o}C), all other solutions and dilutions were freshly prepared.

Extraction was carried out with 50µl of 1.0M Naoh mixed with 200 µl of human plasma and 1.0ml of hexane and ethyle acetate mixture (1:1.v/v) was added, vortexed for 5 minutes. The mixture was centrifuged for 10 minutes and upper organic phase was transferred into second tube, allowed to evaporate to become dry under airstream at ambient temperature. The extract was solubilized in 200 µl of mobile phase.

Chromatographic conditions

Chromatography was carried out by using Isocratic Elution at room temperature. High Pressure liquid chromatography system (Pump LC- 10 AT VP; detector SPD -10AVP, Shimadzu Corp., Kyoto Japan), Communication Bus module (CBM-102), Bonda pack C18, 250, 10µm Germany attached with Guard column. Soft ware LC 10 (Shimadzu Corp., Kyoto, Japan), Ultra Sonic bath (Clifton, Nickel, England), Centrifuge machine (Hereues Germany), Vortex Mixer (Whirl mixer, England), Filtration assembly (Sartorius Germany), Swinney Filtration assembly (Millipore, England). Micropippete (Mettler Toledo, England), Micro liter syringe (Hamilton, Switzerland) and pH meter (Mettler Toledo, England) were used in the analysis.

Validation of assay

Calibration curves (linear) for dimenhydinate in human plasma were drawn for the validation of the assay. Three different standard calibration curves of dimenhydinate were plotted on one day to achieve intraday accuracy and precision. Other two standard curves for calibration of dimenhydinate were plotted on two different days to assess the variation. Quality control (QC) samples at Low, Medium and High concentration levels on three different days were estimated to achieve intraday and inter day accuracy. The Validation procedure was conducted according to International Conference of Harmonization guidelines (ICH, 2005).

Recovery

The extraction of dimenhydrinate from plasma samples was quantified. The recovery of various concentrations was established by differentiation & comparison of peak areas accomplished by plasma samples with drug and blank mobile phase samples.

Selectivity

Selectivity is mandatory to distinguish between drug and other components present in the prepared sample, different samples were run to reveal selectivity.

Long term stability and room temperature stability

Long term stability and room temperature stability are obligated for satisfactory estimation of the procedure's accuracy. Five samples of low and high concentrations were run at different time intervals.

Freeze - thaw stability

Freeze-thaw stability was conducted for Dimenhydrinate in plasma. This stability was estimated with low

concentration 24ng/ml and high concentration 180ng/ml. A number of 20 samples bearing each concentration were frozen at -20^{o}C for a period of 24 hours. A sample of each concentration was defrosted and analyzed.

Other remained samples were kept frozen for further 24 hours. Again next two sets of five different samples of each concentration were assessed in the similar manner and the remaining set was defrosted for another day assessment, in this manner freeze thaw cycles were completed thrice. These samples analyzed with newly prepared samples were used as reference.

RESULTS

Chromatography

The Chromatographic Peak for dimenhydrinate eluted at 4.9 minutes (fig. 1). There were no peaks occurred for blank human plasma. Human plasma samples spiked with dimenhydrinate displayed a satisfactory separation. The total run time for dimenhydrinate was 8 minutes (figs. 2 and 3).

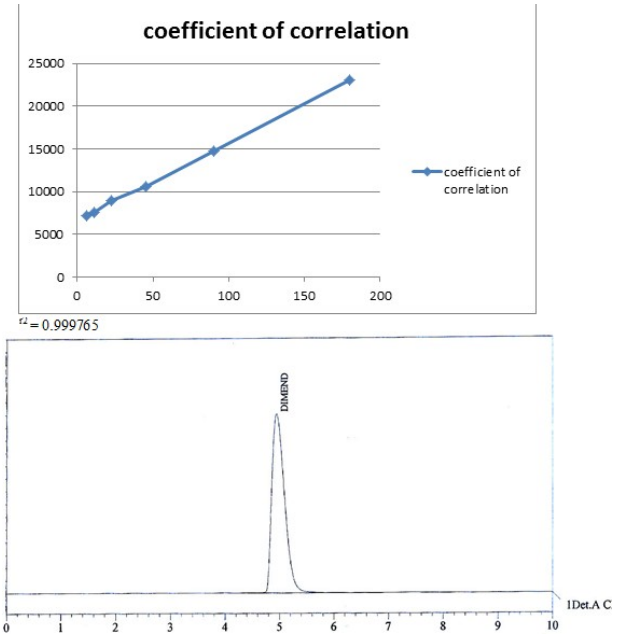


Fig. 1: Dimenhydrinate in Mobile Phase

Linearity

A satisfactory linear relationship between dimenhydrinate in blank plasma and in mobile phase was ascertained, the range of concentration was 6ng/ml to360ng/ml.

Accuracy and precision

The interday and intraday precision and accuracy of HPLC assay method were satisfactory. The intra day and inter day accuracy were found in the range of 89%and 96%. The intra day precision (coefficient of variance) was not more than 0.02 and inter day precession was found not more than .010 table 2.

Limit of detection and limit of quantification

The limit of detection of dimenhydrinate was 2ng/ml, and limit of quantification of dimenhydrinate was 6ng/ml as shown in table 3, fig. 4. The results were comparable with method validation of dimenhydrinate in human plasma on LCMS (Tavares *et al*., 2007).

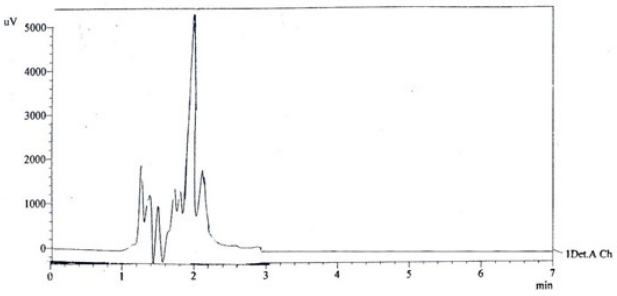


Fig. 2: Blank plasma

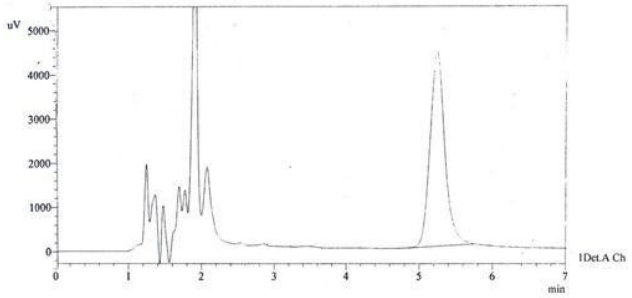


Fig. 3: Dimenhydrinate in human plasma

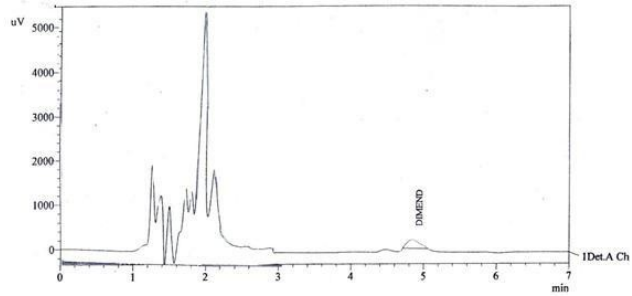


Fig. 4: Lower limit of quantification of dimenhydrinate in human plasma

Recovery

The mean recovery of dimenhydrinate from Human plasma was 97.02% (n=5), it indicates that minimum quantities of dimenhydrinate were lost in Plasma extraction step table 4.

Freeze thaw stability

Freeze - Thaw plasma stability of drug were established for three cycles and assessed % accuracy were found to be 96.66%, 95.0%, 95.41% for low concentration and 98.44%, 98.50%and 94.61% for high concentration table 7.

Selectivity

Blank Plasma Samples (six) were run and analyzed, at the time of Drug retention no other interfering peak was

Table 1: System suitability parameters

Parameter	Mean (n=5)	Relative standard deviation (%)	Limit
Retention time (min)	5	-	Less than 2
Area	30961.8	0.91	Less than 2
Theoretical Plates	8396	0.98	Less than 2
Tailing Factor	1.41	0.87	Less than 2

Table 2: Accuracy and Precision of Dimenhydrinate in plasma

Characteristics	Selected concentrations in method validation (ng/ml)			
	6	18	180	288
	Intraday			
Mean (n=5)	5.71	16.07	172.10	279.06
% Accuracy	95.1	89.2	95.6	96.89
Standard deviation	0.110	0.145	0.07	0.06
% coefficient of variance	1.9	0.90	0.04	0.02
	Inter day			
Mean (n=5)	5.32	16.01	167.33	
% Accuracy	88.6	88.9	92.96	93.26
Standard deviation	0.008	0.005	0.054	0.054
% coefficient of variance	0.017	0.006	0.015	0.010

Table 3: Limit of detection of Dimenhydrinate in plasma

Conc. (ng/ml)	Calculated concentration (ng/ml)					Mean	Standard deviation	% coefficient of variance	% accuracy
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5				
6	5.91	5.90	5.82	5.87	5.71	5.84	0.081	1.39	97.36%
3	2.29	2.30	2.29	2.32	2.30	2.30	0.012	0.52	76.66%
2	1.29	1.30	1.31	1.32	1.28	1.30	0.015	1.15	65.5%
1	-	-	-	-	-	-	-	-	-

Table 4: Result of recovery studies

S/No	Conc. (ng/ml)	Mean peak area in plasma (n=5)	Mean peak area in mobile phase (n=5)	% recovery
1	45	3632.4	3767.9	96.40
2	90	42751.2	43809.6	97.58
3	180	46501.9	47891.5	97.09
Mean Recovery 97.02				

observed. No interference was found for the sample of drug run in the similar conditions. Thus a good selectivity was claimed.

Long term and Room temperature stability

Long term stability and room temperature stability were found by different drug plasma samples run at different time intervals and at different temperatures. The results were acceptable as shown in the tables, 5- 6.

DISCUSSION

The data gathered and presented permits detection of dimenhydrinate in a range of concentrations and the method involves Liquid Liquid extraction (Tavares *et al.*, 2007) and Pharmacopoeial method (United States Pharmacopoeia, 2011).

The present HPLC method has some added advantages , for example small scale requirements, simple process of sample preparation , good sensitivity, ease of handling , in comparison with other methods (Tavares *et al.*, 2007; Ozkan *et al.*, 2013; Barbas *et al.*, 2000 and Doge and Egar., 2007) HPLC-tandem mass spectrometry (LC-MS-MS) HPLC fluorescence detection and HPLC with UV detection. A quantification range of 0.5 to 200ng/ml was reported for diphenhydramine in human plasma on HPLC/TMS (Cathy *et al*., 2017).

Limit of quantification of another H_1 receptor antagonist epinastine in biological fluids was found 2ng/ml by HPLC-MS (Sarashina *et al*., 2005). This method of analysis has improved selectivity which is very critical for pharmacokinetic description of dimenhydrinate clearance inside the body. Preparation of samples of plasma was a single step procedure of extraction with hexane eehyle

Table 5: Room temperature stability

Low concentration 24ng/ml				High concentration 180ng/ml		
Parameter	0hrs	6hr	12hr	0hrs	6hrs	12hrs
Mean(n=5)	24.2	24.0	23.8	180	179.9	177.48
Standard deviation	0.11	0.001	0.19	0.25	0.19	0.26
% Coefficient of variance	0.45	0.83	0.79	0.13	0.10	0.14
% Accuracy	100.83	100	99.16	100	99.94	98.61

Table 6: Long term stability

Parameter	Low concentration 24ng/ml			High concentration 180ng/ml		
	Fresh	2 weeks	3weeks	fresh	2weeks	3weeks
Mean (n=5)	24.1	23.6	22.5	182.0	170	172
Standard deviation	0.143	0.015	0.487	0.158	0.158	0.187
%coefficient of variation	0.59	0.06	2.16	0.086	0.092	0.108
% Accuracy	100.5	98.33	93.75	101.11	94.28	95.55

Table 7: Freeze thaw stability

	24ng/ml				180ng/ml			
	Fresh sample	FT CYCLE 1	FT CYCLE 2	FT CYCLE 3	Fresh sample	FT CYCLE 1	FT CYCLE 2	FT CYCLE 3
Mean (n=5)	23.7	23.2	22.8	22.9	177.2	172.1	177.3	170.3
Standard deviation	0.02	0.015	0.015	0.015	0.158	0.083	0.025	0.025
Coefficient of variance	0.08	0.06	0.069	0.065	0.089	0.048	0.014	0.014
%Accuracy	98.75	96.66	95.0	95.41	98.44	95.61	98.50	94.61

acetate mixture, after wards the samples were centrifuged, air dried and reconstituted with mobile phase before injecting in the column of HPLC instrument. We consider the step is essential for optimum chromatographic results.

Accuracy was expressed as percentage from the theoretical variance along with mean of observed QC samples less, high and medium concentration. It has an enhanced drug recovery rate. High priced instrumentation and cost of operations of LCMS makes it less favorable for analysis, present HPLC method with UV detection seems most suitable for preclinical Bioequivalence studies. The present method can be use successfully for the estimation of dimenhydrinate in mobile phase and Plasma in low concentrations.

CONCLUSION

Simple, unique, fast and reproducible method for the quantitative analysis of dimenhydrinate in Human plasma has been investigated and met the validation criteria well. This bio analytical method is appropriate for analyzing the *in vivo* characteristics of dimenhydrinate after oral administration and give more accurate and precise results.

REFERENCES

Andrea MR, Dandagi PM and Gadad AP (2016). Formulation and Evaluation of fast dissolving buccal films of dimenhydrinate. *Indian Drugs*, **53**(9): 34-41.

Barbas C, García A, Saavedra L and Castro M (2000). Optimization and validation of a method for the determination of caffeine, 8-chlorotheophylline and diphenhydramine by isocratic high-performance liquid chromatography. Stress test for stability evaluation. *J. Chromatogr. A.*, **870**(1-2): 97-103.

BP (2010). British Pharmacopoeia, Her Majesty's Stationery Office, London, 507-508, 703-704, 2620.

Canada MJA, Reguera MIP, Díaz AM and Capitan-Vallvey LF (1999). Solid-phase UV spectroscopic multisensor for the simultaneous determination of caffeine, dimenhydrinate and acetaminophen by using partial least squares multicalibration. *Talanta*., **49**(3): 691-701.

Cathy k Gellote, Brenda A Zimmerman and Gary A Thompson. (2017). Single- dose pharmacokinetic study of diphenhydramine Hcl in children and Adolescents. *Clin. Pharmacol. Drug. Dev.*, pp.1-8.

DiGregorio D, Westgate E and Sherma J (2000). Analysis of the active ingredient dimenhydrinate in motion-sickness tablets by high-performance thin-layer chromatography with ultraviolet absorption densitometry. *Acta. Chromatogr*., **10**: 190-194.

Doge U and Egar K (2007). A simple HPLC-UV method for the determination of dimenhydrinate and related substances identification of an unknown impurity. *Pharmazie.,* **62**(3): 174-178.

EL-Kafrawy DS and Belal TS (2016). Validated HPTLC method for the simultaneous determination of

Cinnarizine and Dimenhydrinate in their combined dosage form. *J. Assn. Arab. Univ. Basic. Appl. Sci. ens.,* **19**: 15-22.

Girish BV, Praveen S and Kathyayini N (2016). Analytical quality by design for development of a sensitive, rapid and stability indicating method for estimation of impurities of dimenhydrinate in its pharmaceutical formulation. *Res. J. Pharm. Biol. Chem. Sci.,* **7**(4): 1309-1322.

ICH guideline Q2A (R1); Validation of Analytical Procedures; Text and Methodology (2005).

Kar A and Aniuha GI (1981). Spectrophotometric determination of dimenhydrinate with Reinecke salt. *J. Pharm. Sci.*, **70**(6): 690-691.

Lamie NT and Monir HH (2016). Simultaneous determination of cinnarizine and dimenhydrinate in binary mixture using chromatographic methods. *J. Chromatogr. Sci.*, **54**(1): 1309-1322.

Lamie NT and Yehia AM (2015). Development of normalized spectra manipulating spectrophotometric methods for simultaneous determination of Dimenhydrinate and Cinnarizine binary mixture. *Spectrochim. Acta, - Part A: Mol. Biomol. Spectrosc.,* **150**: 142-150.

Mitic SS, Miletic GZ, Pavlovic AN, Pecev ET and Velimirovic DS (2012). Quantitative estimation of dimenhydrinate in pharmaceuticals and human control serum using ligand-exchange reaction. *Oxidation Communications.,* **35**(4): 856-868.

Moffat AC, Osselton MD and Widdop B (2004). Clark's Analysis of Drugs and Poisons. The Pharmaceutical Press, London, UK, pp.926-927.

Ong CP, Ng CL, Lee HK and Li SFY (1991). Determination of antihistamines in pharmaceuticals by capillary electrophoresis. *J. Chromatogr. A.*, **588**(1-2): 335-339.

Ozkan CK, Taşdemir U, Taş C, Savaşer A, Erol H and Ozkan Y (2013). Determination of dimenhydrinate nasal delivery system in the blood by RP-LC. *Chromatogr*., **76**(21-22): 1521-1525.

Pekoz AY, Erdal MS, Okyar A, Ocak M, Tekeli F, Kaptan E, Sagirli O and Araman A (2015). Preparation and *in vivo* evaluation of dimenhydrinate buccal mucoadhesive films with enhanced bioavailability. *Drug. Dev. Ind. Pharm.,* **42**(6): 1-5.

Sarashina A, Tatami S, Yamamura N, Tsuda Y and Igarashi T (2005). Population Pharmacokinetics of epinastine, a histamine H_1 receptor antagonist, in adults and children. *Bri. J. Clin, Pharmacol.,* **59**(1): 43-53.

Shubietah RM, Abu Zuhri AZ and Fogg AG (1991). Adsorptive stripping voltammetric determination of dimenhydrinate at a hanging mercury drop electrode. *Microchim. Acta.*, **130**(3): 165-171.

Sweetman SC (2009). Martindale-The Complete Drug Reference (36th ed.), The Pharmaceutical Press, London, UK pp.573-576.

Tavares V, Macedo CC, Montanhez L, Barros FAP, Meurer EC, Campos DR, Coelho EC, Calaffati SA and Pedrazzoli Jr J (2007). Determination of dimenhydrinate in human plasma by liquid chromatography-electro spray tandem mass spectrometry: Application to a relative bioavailability study. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci*., **853**(1-2): 127-132.

USP (2011), United States Pharmacopeia (USP 34) 34th edition, United States Pharmacopeial Convention Inc, Rockville, MD, pp.2587-2589.