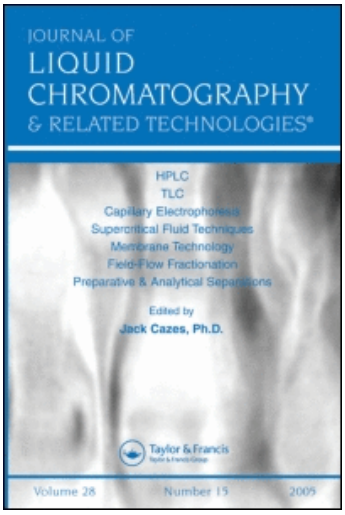

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ebete, K. O. and Koundourellis, J. E.(1997) 'Rapid and Sensitive Procedure for the Quantitation of Pimethixene in Human Milk and Plasma by Solid Phase Extraction (SPE) Using HPLC', Journal of Liquid Chromatography & Related Technologies, 20: 5, 789 — 796
To link to this Article: DOI: 10.1080/10826079708014143
URL: http://dx.doi.org/10.1080/10826079708014143

J. LIQ. CHROM. & REL. TECHNOL., 20(5), 789–796 (1997)

RAPID AND SENSITIVE PROCEDURE FOR THE QUANTITATION OF PIMETHIXENE IN HUMAN MILK AND PLASMA BY SOLID PHASE EXTRACTION (SPE) USING HPLC

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ABSTRACT

A method is described for the quantitative determination of pimethixene from plasma and milk samples by reversed-phase high performance liquid chromatography using diphenylpyraline as an internal standard.

The sample matrix was processed by a solid phase extraction (SPE) technique using 3 mL C_{18} Supelco cartridges. The plasma and milk samples were deproteinized using acetonitrile and the liquid layer was combined with water (1:2 v/v), throughly mixed and subsequently passed through pre-conditioned cartridges. The compounds were eluted with 6 mL of methanol. Then the methanolic solution was evaporated to dryness, reconstituted with 1 mL of methanol and analysed with an octyl-ODP column (150 X 6 mm I.D).

The mobile phase consisted of acetate buffer 0.051 M - methanol (62:38 v/v). Detection was perfomed at 254 nm with the sensitivity set at 0.002 AUFS. The overall recovery of pimethixene was 86.82% in plasma and 80.39% in milk. The limit of detection, corresponding to 2 times signal to noise ratio, was estimated as 15 ng/mL in both plasma and milk samples.

INTRODUCTION

Pimethixene, 9-(1-methyl-4-piperidylidene) thioxanthene, is a potent antagonist of histamine and serotonin. It has the general properties and uses of antihistamines, and has been reported to have sedative properties.[1] Pimethixene has been used in the treatment of respiratory disorders in children[2] and are readily available over the counter under the following proprietary names: calmixene, muricalm and sedosil. Although there are several reports related to HPLC analysis of antihistamines as a class of drugs[3,4,5] but is non-specific for pimethixene. The present report describes a high performance liquid chromatographic procedure for the quantitative determination of pimethixene in milk and plasma samples by solid phase extraction, using diphenylpyraline as the internal standard.

EXPERIMENTAL

Chemicals and Solutions

Pimethixene maleate and diphenylpyraline hydrochloride were supplied by Sigma Company (St. Louis, MO, USA) and used without further purification. Ammonium acetate and glacial acetic acid, analytical reagent grade, were obtained from Merck (Darmstadt, Germany). HPLC grade methanol and water were used throughout.

Stock solutions were prepared by accurately weighing appropriate quantities of pimethixene maleate and diphenylpyraline hydrochloride, and dissolving each, separately, in a 50 mL volumetric flask to prepare a 1 mg/mL solution in methanol. The working standards of pimethixene in the concentration range between 53 and 740 ng/mL, and containing 250 ng/mL of diphenylpyraline as internal standard, were prepared by diluting the appropriate quantities of stock solution with methanol.

Apparatus

A solvent delivery systen with two Model LC-6A high pressure pumps, coupled with a model SPD-6AV UV spectrophotometric detector, operated at 254 nm were controlled by a system programmer, model SCL-6B (Shimadzu Instruments, Kyoto, Japan). The reversed-phase octyl-ODP column (150 X 6 mm I.D), product of Asahi Chemical Company, Kawasaki, Japan, was placed in a model CTO-6A oven at 40°C, and equipped with a Rheodyne 7161 injector fitted with a 20 μL loop, was operated isocratically. Chromatograms were recorded on a chart paper with a Chromatopac model C-R 6A thermal printer-plotter (Shimadzu) at a speed of 2 cm/min. Supelclean C_{18} cartridges and vacuum glass manifold were obtained from Supelco, Bellefonte, PA, USA).

Chromatographic Conditions

The mobile phase was acetate buffer, ionic strength 0.051 M - methanol (62:38 v/v). The pH was adjusted to 3.5 using glacial acetic acid. The column was equilibrated with the eluting solvent by pumping the mobile phase at a flow rate of 0.3 mL/min for 24 h and degassed by slowly bubbling with helium gas throughout the analysis. The flow rate was set at 1.4 mL/min during analysis and the detection performed at 254 nm with a sensitivity of 0.002 absorbance unit full scale (AUFS).

Extraction Procedure

Stock pimethixene methanolic solution, 1 mL, of concentrations ranging from 53-740 ng/mL and containing a fixed amount (250 ng/mL) of diphenylpyraline used as an internal standard was evaporated to dryness in a 15 mL glass centrifuge tube under nitrogen. Plasma or milk sample, 1 mL, and 3 mL of acetonitrile were added into the tubes, vortexed at high speed, briefly, and centrifuged at 2000 rpm for 5 min to precipitate the protein particles. The upper clear layer was collected and transferred to another clean tube. Water, 6 mL, was added to the tube, thoroughly mixed and subsequently treated by solid phase extraction using 3 mL C_{18} Supelco cartridges. The mixture was slowly passed through the cartridges that had been conditioned with 2 mL of methanol and washed with 2 mL of water. The cartridges were fitted to a vacuum glass manifold system that helped to control the flow rate of solvent through the particles. The sorbents were washed once with 2 mL of water before the final elution of the compounds with 6 mL of methanol. It was observed that the addition of 6 mL of water to the sample before processing by solid phase extraction was necessary, as the water helps to hold back the compounds on the

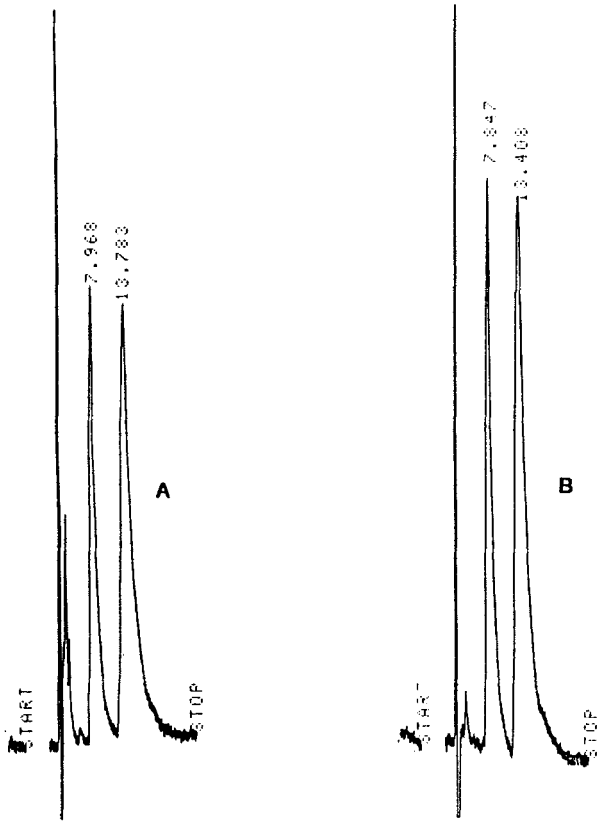

Figure 1. Representative chromatograms of (A) plasma and (B) human milk extracts spiked with pimethixine and diphenylpyraline as internal standard at different concentrations.

particles by reducing the concentration of acetonitrile which has the ability to elute the compounds together with unwanted materials from the packings. Therefore, washing the packings with 2 mL of water helps to remove the unwanted materials while holding back the desired compounds on the packing before the final elution with 6 mL of methanol. The methanolic solution was evaporated to dryness with an evaporation unit (Pierce Reacti-Therm, Model 18780) under a stream of nitrogen at 45°C. The residue was redissolved in 1 mL of methanol and aliquots of 20 μL were injected onto the analytical column.

RESULTS AND DISCUSSION

Figures 1A and 1B represent typical chromatograms of pimethixene and diphenylpyraline extracts from spiked plasma and milk samples processed by solid phase extraction. The retention time of pimethixene was 13.4 min and that of diphenylpyraline 8.0 min, with a resolution of R_s=3.67.

The calibration plot of peak height ratio of pimethixene to diphenylpyraline against concentration of pimethixene, in the range of 53-740 ng/mL was linear. The linear regression and correlation analyses were found to be y=0.0049x + 0.0044 (r=0.9999) and y=0.0046x - 0.0033 (r=0.9998) for pimethixene from plasma and milk samples, respectively.

The recovery efficiency of pimethixene from plasma and milk was determined by making a standard calibration curve of the peak height ratio of pimethixene to the internal standard after direct injection of the methanolic solution containing known quantities of pimethixene (53, 106, 211, 317, 422, 528, 634, and 740 ng/mL) against their concentrations and, by using the characteristics of this standard curve, the recovered amount of the drug after extracting spiked plasma or milk samples containing an equivalent amount of the drug was calculated. At each of the eight pimethixene concentrations used, four replicate samples were measured; results are presented in Tables 1 and 2.

The overall recovery of pimethixene was estimated by plotting a standard calibration of the "added" versus the "found" concentrations of pimethixene in both plasma and milk samples, which gave linear relationships with y=0.8682x + 9.63 (r=0.9996) for plasma and y=0.8039x + 8.33 (r=0.9998) for milk.

Therefore the slopes (0.8682 for plasma and 0.8039 for milk) of these regression lines were used as estimates of the overall recovery for pimethixene, 86.82% for plasma and 80.39% for milk samples, respectively.

Precision and accuracy of the method were assessed by spiking plasma and milk samples with pimethixene standards at two concentration levels (106 and 422 ng/mL) and each containing 250 ng/mL of diphenylpyraline as internal standard. Extraction and HPLC analysis were done as described above. The intra-day precision for each concentration level resulted from four determinations n=4, of samples of same concentration while the overall accuracy and precision for each concentration level resulted from twelve determinations, n=12, of samples of the same concentration at three different days (4X3) within one month.

Table 1

Standard Calibration and Recovery Data of Pimethixene from Spiked Plasma Samples Treated by SPE (n=4)

	Added Conc. (ng/mL)	Mean Peak Ht. Ratio	Found Conc. (ng/mL)	S.D.	C.V. %	Recovery %
1	53	0.268	55.91	0.57	1.02	105.50
2	106	0.521	100.50	0.98	0.98	94.77
3	211	1.056	194.50	1.95	1.00	92.17
4	317	1.578	286.20	2.85	1.00	90.29
5	422	2.085	375.30	3.90	1.04	88.94
6	528	2.592	464.40	4.55	0.98	87.96
7	634	3.141	560.90	5.38	0.96	88.48
8	739	3.662	652.50	6.72	1.03	88.30

$y = 0.0049x + 0.0044$ ($r = 0.999$); Average recovery = 92.05%; Average C.V. = 1.00%.

Table 2

Standard Calibration and Recovery Data of Pimethixene from Spiked Milk Samples Treated by SPE (n=4)

	Added Conc. (ng/mL)	Mean Peak Ht. Ratio	Found Conc. (ng/mL)	S.D.	C.V. %	Recovery %
1	53	0.242	51.50	0.49	0.95	97.13
2	106	0.470	91.40	1.06	1.16	86.26
3	211	0.939	174.00	1.80	1.04	82.46
4	317	1.485	269.90	2.51	0.93	85.14
5	422	1.939	349.70	3.36	0.96	82.86
6	528	2.394	429.60	4.17	0.97	81.37
7	634	2.909	520.20	5.48	1.05	82.04
8	739	3.364	600.10	6.12	1.02	81.21

$y = 0.0046x - 0.0033$ ($r = 0.9998$); Average recovery = 84.81%; Average C.V. = 1.01%.

Table 3

Accuracy and Precision Data for the Determination of Pimethixene from Spiked Plasma Samples

Spiked Amount (ng/mL)	Day	n	Accuracy Found Conc.	Precision S.D.	Precision C.V.%	Overall Mean Conc. ± S.D.
106	1	4	100.50	1.40	1.39	
	2	4	98.77	1.28	1.30	99.33±1.35
	3	4	98.68	1.37	1.39	
422	1	4	378.10	3.98	1.05	
	2	4	376.80	3.91	1.04	376.95±39
	3	4	375.90	4.01	1.07	

Table 4

Accuracy and Precision Data for the Determination of Pimethixene from Spiked Milk Samples

Spiked Amount (ng/mL)	Day	n	Accuracy Found Conc.	Precision S.D.	Precision C.V.%	Overall Mean Conc. ± S.D.
106	1	4	92.08	1.16	1.26	
	2	4	92.16	1.08	1.17	92.0±1.15
	3	4	91.77	1.22	1.33	
422	1	4	350.60	3.77	1.08	
	2	4	351.50	3.56	1.02	350.69±3.66
	3	4	350.00	3.65	1.04	

Therefore for the assessment of the overall precision within one month at two concentration levels, 24 plasma and milk samples each were treated. The results are presented in Tables 3 and 4. The detection limit of the assay, defined as the minimum drug concentration to produce 2 times signal to noise ratio at 0.002 AUFS, was found to be approximately 15 ng/mL for both plasma and milk samples.

CONCLUSION

The technical simplicity, speed and specificity of the method guarantee a reliable procedure for routine analysis of pimethixene in biological fluids.

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Received May 13, 1996
Accepted May 30, 1996
Manuscript 4190