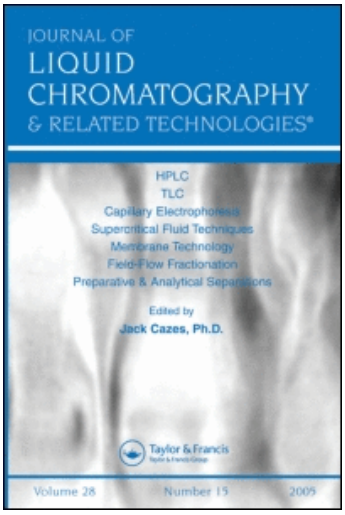

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Simple Reversed Phase Partition Thin Layer Chromatographic Method for Rapid Identification and Quantitation of Methotrexate in Presence of its Disintegration Products

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To cite this Article Datta, K. , Roy, S. K. and Das, S. K.(1990) 'A Simple Reversed Phase Partition Thin Layer Chromatographic Method for Rapid Identification and Quantitation of Methotrexate in Presence of its Disintegration Products', Journal of Liquid Chromatography & Related Technologies, 13: 10, 1933 — 1941

To link to this Article: DOI: 10.1080/01483919008049002

URL: http://dx.doi.org/10.1080/01483919008049002

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(10), 1933-1941 (1990)

A SIMPLE REVERSED PHASE PARTITION THIN LAYER CHROMATOGRAPHIC METHOD FOR RAPID IDENTIFICATION AND QUANTITATION OF METHOTREXATE IN PRESENCE OF ITS DISINTEGRATION PRODUCTS

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SUMMARY

A rapid, sensitive, inexpensive and specific method for identification and quantitation of methotrexate present in pharmaceutical dosage forms or in bulk drug is described. Methotrexate is completely separated from its disintegration products by reversed phase thin layer chromatography on silanized silicagel HF 254. The solvent system being citrate phosphate buffer pH 6.0 : methanol :: 70 : 30. The buffer contained 4.62% of disodium hydrogen phosphate and 0.7% citric acid. The plate was observed under short wave UV and methotrexate was assayed either by dual wave length densitometry at 302 nm with a background correction at 333 nm or by measuring optical density at 302 nm after extraction from the plate with methanol : 0.1 N HCl :: 1 : 1 by conventional method. Superiority of the proposed method over HPLC and paper chromatographic methods is described.

INTRODUCTION

Methotrexate, a cytotoxic folic acid antagonist is widely used now-a-days in cancer chemotherapy (1). The drug is sensitive to heat and light and methotrexate available as bulk material or as pharmaceutical preparations are often contaminated with degradation products and analogues (1,2). Since the usual dose of methotrexate is high and sometimes as much as 10-15 g/day are administered to patients (1,3), it is necessary that these contaminants be detected and methotrexate be assayed accurately in the dosage unit. Methotrexate is usually analysed by paper chromatography (PC) (4), or high performance liquid chromatography (HPLC) (3,4,5). However the PC method is time consuming and the HPLC method, though rapid and specific, has a high operational cost (6) and the average time required for assay of a single sample is about one hour (running time)(5) which makes the method unsuitable for analysis of a large number of samples at a time. In this paper, we have reported a simple and inexpensive but specific reversed phase partition thin layer chromatography (RPTLC) method suitable for analysis of a large number of samples accurately.

METHOD

Apparatus

TLC Scanner - Shimadzu dual wave length zigzag scanner, model CS-930.

Spectrophotometer - Shimadzu UV-VIS dual beam recording spectrophotometer model Graphicord UV 240 with OPI 4.

HPLC System - Waters HPLC system equiped with model 510 HPLC pump, model 6U K universal injector, model 440 UV/VIS detector and Datamodule 745 recording integrater with 30cm X 0.4cm μ Bondapack C-18 column.

Ultraviolet Viewer - Desaga UVIS system.

Sample applicator - Camag Nanomat II with 1 μl and 20 μl micropipette with holder.

Table centrifuge - Model Remi 8R with swing head, 5000 rpm.

TLC plate - 20 X 20 cm coated with 0.4 mm thick layer of silanized silica-gel, E. Meck (60 H : 60 HF 254 ::1:1 w/w)

Reagents

Methotrexate test samples (methotrexate injection or tablets) were bought from local market, bulk drug of methotrexate was received in our laboratory for testing and reference standard of methotrexate was of USPRS grade (5).

Solvents for TLC were of G.R. E. Merck grade and used as such. Solvents for HPLC were of Lichrosolve E. Merck grade. Buffer solution contained 4.62% disodium hydrogen orthophosphate and 0.7% citric acid, pH 6.0.

Mobile phase

1) TLC - Citrate phosphate buffer : methanol :: 70 : 30 v/v.

2) HPLC - Citrate phosphate buffer : acetonitrile :: 90 : 10 v/v. (5)

Standard preparation

Methotrexate R.S. (50 mg) was dissolved in 25 ml of 0.01 N NaOH and the volume was made up to 50 ml with methanol.

Sample preparation

Bulk drug : solution for bulk drug was prepared as described under "Standard".

Parental preparation : The sample was diluted with 0.01N NaOH to a methotrexate concentration of 2 mg/ml which was further diluted with methanol to a final concentration of 1 mg/ml.

Tablets : Twenty tablets were finely powdered and the powder equivalent to 50 mg of methotrexate was shaken on a mechanical shaker for 10 min. with 25 ml of 0.01N NaOH and centrifuged. The clear supernatant (10 ml) was diluted with methanol to a final concentration of 1 mg/ml.

The standard or the sample solution should not be stored.

Acid and alkali degradation products

Methotrexate (4 mg) in 1 ml of 2(N) HCl or 1(N) NaOH was heated in a boiling water bath for 30 min., cooled and diluted with methanol to 2 ml.

Heat and light degradation products

Heat and light degradation products were prepared as suggested by Chatterjee & Gallelli (3). Methotrexate (10 mg) was dissolved in 5 ml of 0.1 M ammonia-ammonium bicarbonate buffer pH 8.3, taken in an ampule, sealed and heated at 85°C for 10 days (heat degradation) or kept under a fluorescent light at room temperature for 1 week (light degradation). The ampules were then opened and the contents were diluted to 2 volumes with methanol.

PROCEDURE

For assay by scanning densitometry 1 µl of the test or standard preparations were applied as compact spots 10 mm apart on an imaginary line 15 mm from one edge of the TLC plate. The spots were dried in a current of air. The plate was developed upto 50% of the total length of the plate in usual way in filter paper lined tank previously saturated with mobile phase for 1 h and containing 15 ml of the mobile phase in each trough. After development the plate was dried in a current of air and methotrexate (or the contaminants) spots were visualised under short wave ultraviolet (UV) lamp and identified by comparing with Rf value of the standard. The plate was then scanned in the densitometer with linearizer setting at X = 3, back ground scanning wave length at 333 nm and sample scanning wave length at 302 nm. Other parameters were set as was required for the chromatogramme.

To study the linearity of area values with concentration, standard solutions containing 0.5 mg/ml to 8 mg/ml was estimated by the above method.

To study the solvent suitability and resolution, the disintegration products prepared were chromatographed and documented by densitometric method.

For spectrophotometric assay after TLC, 20 µl of the standard and the sample solution were applied as separate 5 mm wide bands 15 mm apart on the

TABLE 1

Assay value of Methotrexate (percent label claim) present in Pharmaceutical dosage forms or in bulk drug obtained by the proposed and official methods

Sample Number[a]	Proposed method[b]				Official method[b,c]	
	TLC				HPLC	
	Densitometry		Spectroscopy			
	Average	SD[d]	Average	SD	Average	SD
1.	111.16	1.32	112.10	1.36	112.52	1.96
2.	108.47	1.52	107.90	0.54	107.41	1.66
3.	109.68	1.36	109.60	0.00	110.45	1.95
4.	110.00	1.88	109.20	0.82	108.96	1.34
5.	100.00	1.65	100.00	1.65	100.00	1.41
6.	98.52	1.80	98.62	1.08	98.10	1.80
7.	106.04	2.07	106.59	1.74	106.19	1.04
8.	101.07	1.48	100.48	0.96	100.87	0.31

a. 1,2 and 3,4 were injections (25 and 12.5 mg per ml), 5 and 6 bulk drug received for testing and 7 & 8 tablets (2.5 mg per tablets)

b. Average of 6 independent determinations

c. USP XXI (5)

d. Standard deviations.

TLC plate which was then developed and visualised as above. The methotrexate bands were marked and scrapped into separate 15 ml stoppered glass centriguge tubes. Methotrexate was then extracted from silicagel by shaking with 2 X 2 ml of 0.1 N HCl : methanol :: 1 : 1 on a vortex mixer for 30 sec. followed by centrifugation each time at 1000 g for 5 min. Optical density of the combined extract of each sample were measured at 302 nm. A blank obtained by extraction of the adsorbent from an area equivalent to respective sample bands but not containing any sample was used in the reference beam.

TABLE 2

Linearity of concentration of methotrexate with area values obtained by densitometry after TLC

Amount applied (µg)	Integrated area value		r	b	a
	Average*	SD			
0.5	49929	987	0.9998	0.9988	-0.01296
1.0	96473	1171	($p \ll 0.001$)		
2.0	195893	2610			
4.0	395640	4464			
8.0	705392	26718			

*Average of 6 independent determinations.

For HPLC, the method described by the USP XXI (5) was followed.

RESULTS AND DISCUSSION

The proposed method was validated by comparing the results with those obtained by an official method (5). Table 1 shows assay results of methotrexate present in different pharmaceutical preparations and in bulk drug, obtained by the proposed and official methods. There is a good agreement between the results obtained by all the methods. Table 2 indicates the relations of quantity of sample applied with integrated area value obtained by densitometry after TLC. The 'r' value (0.9998) indicates significant linear relationship under the experimental conditions (amount applied : 0.5 to 4.0 µg). Also a very low 'a' value (-0.01296)

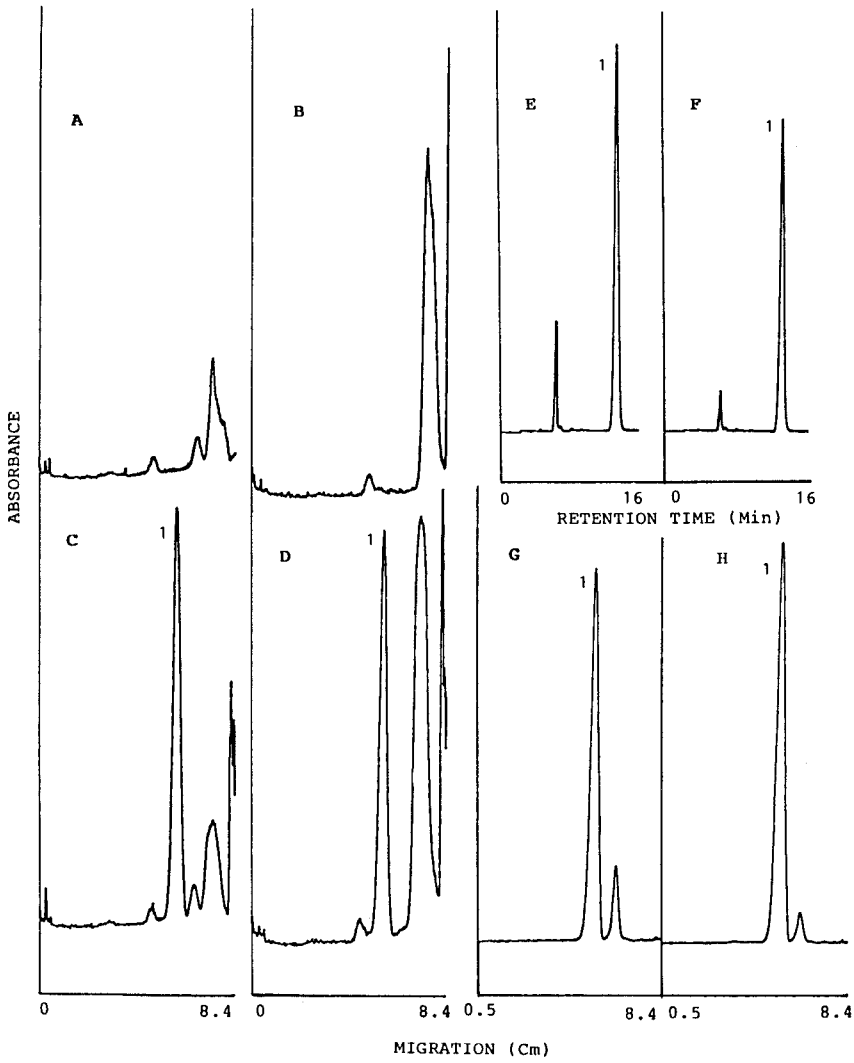

Fig-1 Separation of methotrexate from its degradation products.
A - Acid hydrolyzate, B - alkali hydrolyzate, C - A + methotrexate D - B + methotrexate, E & G - Heat degradation products, F & H. Light degradation products. E & F. HPLC, others RPTLC. 1 - Methotrexate peak.

TABLE 3

Assay values (percent composition) of methotrexate and its heat and light degradation products by proposed[a] and HPLC methods.

Compound	Heat degradation				Light degradation			
	Proposed		HPLC		Proposed		HPLC	
	Average[b]	SD	Average	SD	Average	SD	Average	SD
Methotrexate	87.5	0.52	88.1	0.10	94.5	0.61	94.3	0.01
Degradation Products	12.5	0.32	12.0	0.12	5.5	0.11	5.7	0.02

a. TLC followed by densitometry

b. Average of 4 independent determinations

shows that the locus practically passes through the origin. However, at higher concentrations (8 μg and above) the liniarity may not be maintained.

The reflectance spectrum of methotrexate showed absorption maxima at 302 nm and the absorption was negligible in comparison at 333 nm. So we used 302 nm for scanning and 333 nm for background correction (for laboratory drawn TLC plates). However, for precoated plates or uniformly drawn plates, this background correction is not necessary.

Assay of methotrexate by spectroscopy after TLC gives uniform results with a very low standard deviation value (Table 1). This method is very useful where a good densitometer is not available or where very accurate results are required. However, this method is more laborious than the densitometric assay and is not as fast.

Assay of methotrexate after chromatography on cellulose (PC or TLC) was recommended by some leading pharmacopoeia (4,7). PC is still in use (4). However, PC is time consuming and we could not separate methotrexate completely from its breakdown products by TLC on cellulose layers using different solvent systems (4,7). However, The RPTLC method developed by us completely separates heat or light degradation products from methotrexate (Fig. 1). Though the resolution factor is not as high as HPLC, composition determined by the TLC densitometric method is the same as those obtained by the HPLC method (Table 3). Methotrexate is completely separated from its acid or alkali degradation products also by the method described (Fig. 1).

The proposed TLC method for assay of methotrexate is as precise as HPLC method but is inexpensive, rapid and capable of handling a large number of samples at a time. The method is ideal for drug quality assurance laboratories. Because of the simplicity and sensitivity, the method is also suitable for medical institutions who use this medicine.

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