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DEVELOPED HPTLC-DENSITOMETRIC FOR THERAPEUTIC DRUG MONITORING

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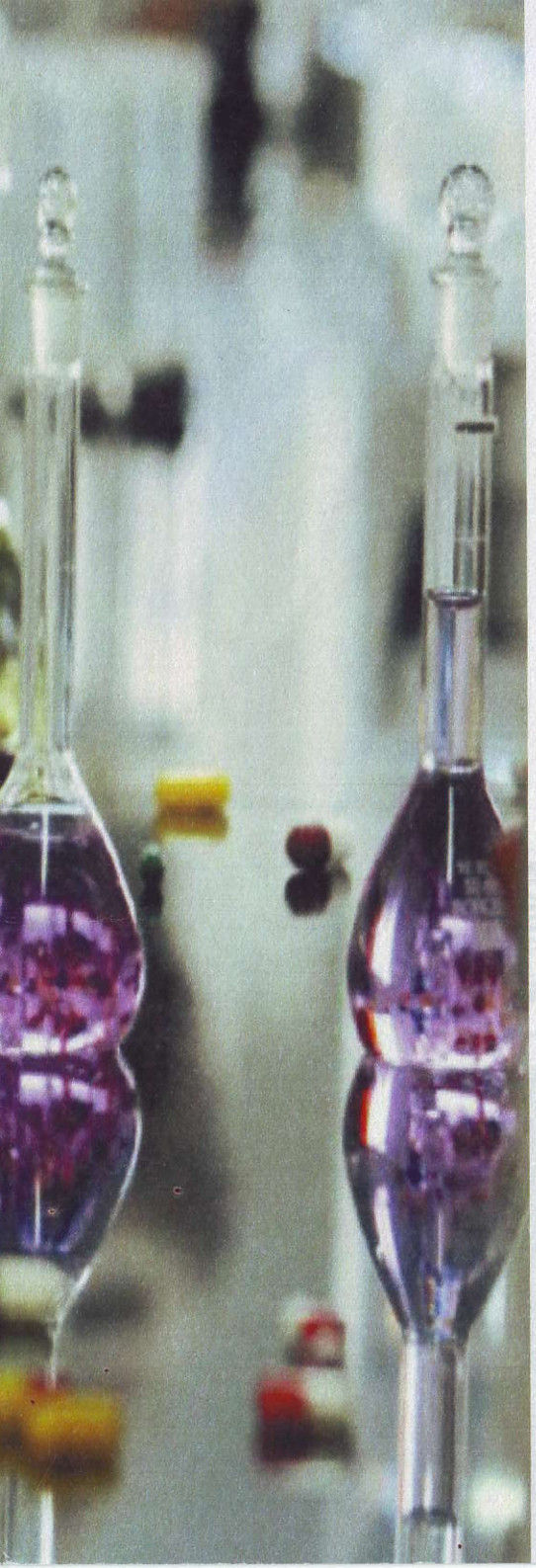
ABSTRACT

Therapeutic drug monitoring (TDM) supported carrying out of patient safety program in hospital. One factor can be restricted an application of TDM in a small hospital is lack of existence of analytical instrumentation. HPTLC-densitometric is one of inexpensive, robust, and easy to implemented analytical method. We developed the use combination of HPTLC-densitometric and multiple use of Solid Phase Extraction (SPE)-cartridge for TDM. In the first study we used phenytoin as an analytical target and SPE-C18 for extraction. Phenobarbital was used as internal standard, HPTLC Si_{60} GF_{254} as stationary phase and mobile phase was combination of ethyl acetate-methanol-ammonia, 85:10:5 v/v/v. The drug extracted from plasma by multiple using of one SPE-cartridge and methanol-acetonitrile (2:3, v/v) as eluent. Phenytoin concentrations linear were within range 100-3200 ng per spot with LOD and LOQ 146.327±1.669 ng and 487.758±5.563 ng respectively. Ten times using of one SPE-cartridge for phenytoin extraction still gave better recovery. Application this method can reduce the analytical cost.



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Developed HPTLC-Densitometric for therapeutic drug monitoring

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Therapeutic drug monitoring (TDM) supported carrying out of patient safety program in hospital. One factor can be restricted an application of TDM in a small hospital is lack of existence of analytical instrumentation. HPTLC-densitometric is one of inexpensive, robust, and easy to implemented analytical method. We developed the use combination of HPTLC-densitometric and multiple use of Solid Phase Extraction (SPE)-cartridge for TDM. In the first study we used phenytoin as an analytical target and SPE-C18 for extraction.

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Introduction

TDM is defined as measuring serum concentrations of a drug in a single or multiple time points in a biological matrix after a dosage, with appropriate interpretation, will directly influence prescribing procedures. TDM has clinical importance for drugs with a narrow therapeutic window. TDM has reported can increasing of safety of patients, decrease hospital stay and has important implications on the cost of medical care.

Phenytoin is one of controlled antiepileptic drug. Phenytoin is 90% bound to serum proteins, mainly albumin. Its therapeutic arranges concentration is 0.8-2.1 µg/mL. Free phenytoin concentration correlated better with pharmacological effect or toxicity.

Different types of assays are used in clinical laboratories for determination of concentration phenytoin in serum for TDM. For analysis of this durg, GC, HPLC, or HPLC comined with tandem mass spectrometric techniques are used. Fenimore et al. (1978) reported the usage of high-performance thin layer chromatography (HPTLC) to determine anticonvulsant durgs phenobarbaital and phenytoin, which frequently administered to patients receiving treatment at mental-health facilities. The low of limit detection of HPTLC-densitometric technique, is possible to need little amount of biological sample.

In view of this, HPTLC based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution.

Method

Reagents-Stock solution Preparation

HPTLC Method and Chromatographic Conditions

Sample Application: *Drugs were spotted on Precoated plates in the form of narrow bands of lengths 6 mm, with 10 mm from the bottom and left margin and with 10mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas.*

Mobile Phase selection and Migration: *Plates were developed using mobile phase consisting of a) toluene-asetone (5.0:2.0, v/v), b) chloroform-acetone (8.0+2.0, v/v) c) ethyl acetate-methanol-con.ammonia (85.0 + 10.0 + 5.0 v/v/v). Linear ascending development was carried out in 10 cm x 10 cm twin trough glass chamber equilibrated with mobile phase.*

Densitometric Analysis and Quantitation Procedure:
Densitometric scanning was performed on Camag TLC scanner III in absorbance mode and operated by winCATS version 1.4.3.8121. The spots were analyzed at a wavelength of 200 nm. The slit dimensions used in the analysis were 6.00mm x 0.30 mm, with a scanning rate of 20 mm/s, and data resolution 100 µm/step

Extraction (LLE & SPE):
Each tube was added 100 µL plasma, every 3 tubes were added 10 µL of phenytoin (50, 200, and 250 ng/µL), each tubes were added 16 µL IS (50ng/µL). Protein was precipitated by add 200 µL acetonitrile, tubes were capped, then centrifuge at 6000 rpm for 15 minutes. Supernatant obtained then extracted saparately.
LLE
A obtained supernatant was added of 1 mL ethyl acetat was vortex-mixed. The organic phase was evaporated to dryness at 80 oC and residue dissolved in 25 µL methanol.
SPE
Extraction was performed with a vacuum manifold assembled with C-18 chomophob columns. The column was preconditioned with 5 mL acetonitrile, followed by 10 mL aquadest. The vacuum was turned off. Pretreated samples were transferred to the column. The column was rinsed by passing though it sequentially: (a) 1 mL of phosphat buffer pH 5.7, then the column was dried under full vacuum, (b) 20 mL aquadest, and the column was once again dried under full vacuum, (c) then eluted with 5 ml methanol-acetonitrile (2:3, v/v). The column was released from the manifold and 5 mL of eluat were passed through it and collected. Organic phase was then evaporated and reconstituted in 25 µL methanol.

Method Validation

Result

Table 1 Linear regression data for the calibration curves and LOD LOQ (n = 3)

Plat	Linear regression	r^2	*LOD* (ng/spot)	*LOQ* (ng/spot)
1	y = 0.0008x + 0.1790	0.999	145.67	485.57
2	y = 0.0008x + 0.1812	0.999	145.08	483.62
3	y = 0.0008x + 0.1794	0.998	148.22	494.08
average		0.999	146.32	487.76
SD		0.001	1.67	5.56
(%) RSD		0.058	1.14	1.14

Table 2. Intraprecision studies (n = 3)

Amount spotes Drug (ng/spot)	Area Peak ratio Phenytoin to IS (Mean ± SD)	RSD (%)
400	0.459 ± 0.009	1.88
800	0.844 ± 0.002	0.18
1600	1.429 ± 0.001	0.07

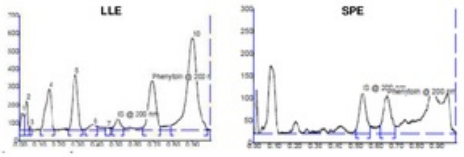

Figure 1. Chromatogram of standard phenytoin and IS: phenobarbital after extracted (LLE and SPE) using mobile phase: ethyl acetat-methanol-con. ammonia (85+10+5, v/v/v)

Table 3. Recovery *LLE intra- and interday* studies

Amount of drug (ng/spot)	Average recovery ± SD	(%) RSD
Intraday (n=3)		
500	92.70 ± 5.54	5.97
2000	93.91 ± 4,71	5.01
2500	105.91 ± 3.05	2.88
Interday (n=3)		
500	95.80 ± 2.86	2.98
2000	90.01 ± 3.52	5.91
2500	91.90 ± 12.36	13.45

Table 4. Recovery *SPE-C18 intra- and interday* studies

Amount of drug (ng/spot)	Average recovery ± SD	(%) RSD
Intraday (n=3)		
500	97.99 ± 1.02	1.04
2000	97.68 ± 1.42	1.45
2500	96.41 ± 1.46	1.51
Interday (n=3)		
500	100.26 ± 1.99	1.99
2000	99.82 ± 3.82	3.82
2500	100.68 ± 4.21	4.18

Summary

Various solven systems such a) toluene-asetone (5.0:2.0, v/v), b) chloroform-acetone (8.0+2.0, v/v), and c) ethyl acetate-methanol-con.ammonia (85.0 + 10.0 + 5.0 v/v/v) were evaluated. Among these, solvent system c good separation of phenytoin from I.S. Under the experimental conditions employed, the LOD of durg that could be detected was found to be .146.32 ng/spot and the LOQ of drug was found 487.76 ng/spot, with RSD < 5%. Method was found to be linear in a concentration range of 200- ng/spot (n=3), with respect to a peak area. Statistic analysis of recovery result between LLE and SPE showed not singnificant differences. But SPE gave more clear profile chromatogram then LLE (figure 1). Based on LOQ, range of linearity of drug, range of therapeutic of phenytoin and validation result, this method can be used to determinete Phenytoin in propuse TDM.

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