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Stability-Indicating High-Performance Liquid Chromatographic Analysis of Lidocaine Hydrochloride and Lidocaine Hydrochloride with Epinephrine Injectable Solutions

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Abstract □ A reversed-phase, high-performance liquid chromatographic (HPLC) procedure, which is specific and quantitative for lidocaine hydrochloride, epinephrine, and methylparaben, was developed for the analysis of lidocaine hydrochloride and lidocaine hydrochloride with epinephrine solutions for injection. Epinephrine sulfonic acid and adrenochrome are separated in this system. Also separated are lidocaine and methylparaben and their respective degradation products, 2,6-xylidine and *p*-hydroxybenzoic acid. The analysis requires that three detectors (two UV and one electrochemical) be connected in series. By using this arrangement, lidocaine hydrochloride and methylparaben are quantitated by UV at 254 and 280 nm, respectively, while epinephrine is quantitated electrochemically. The method is simple, accurate, precise, and rapid. No sample preparation or internal standard is necessary, and only a 2-μl sample volume is required for analysis. Chromatographic conditions include a μBondapak CN column and a mobile phase of 0.01 *M* 1-octanesulfonic acid sodium salt, 0.1 m*M* edetate disodium, 2% acetic acid, 2% acetonitrile, and 1% methanol in water.

Keyphrases □ High-performance liquid chromatography—specific and quantitative stability-indicating procedure for lidocaine hydrochloride, epinephrine, and methylparaben □ Lidocaine hydrochloride—injectable solutions of epinephrine using stability-indicating high-performance liquid chromatography □ Epinephrine—high-performance liquid chromatographic procedure for assaying components of injectable solutions □ Methylparaben—high-performance liquid chromatography for assaying components of injectable solutions

A literature review indicated that the various colorimetric and fluorometric methods utilized for the analysis of epinephrine in local anesthetic solutions all have problems associated with them (1, 2). The colorimetric methods generally are not stability indicating. Epinephrine sulfonic acid and bisulfite interfere with the development of the color, resulting in a lack of specificity for intact epinephrine.

A fluorometric procedure, based on the trihydroxyindole reaction first observed by Loew (3) and later modified (4, 5), is specific for epinephrine but is subject to many variables such as time, temperature, pH, presence of bisulfite, and the composition of the final alkaline/ascorbate reagent. The current USP (6) method for assaying epinephrine in lidocaine hydrochloride injectable solutions is a fluorometric procedure; although the method is specific for epinephrine, its application requires a great deal of experience and technique. The fluorometric procedure for the analysis of epinephrine in lidocaine hydrochloride injectable solutions has been automated (7) but is subject to interferences.

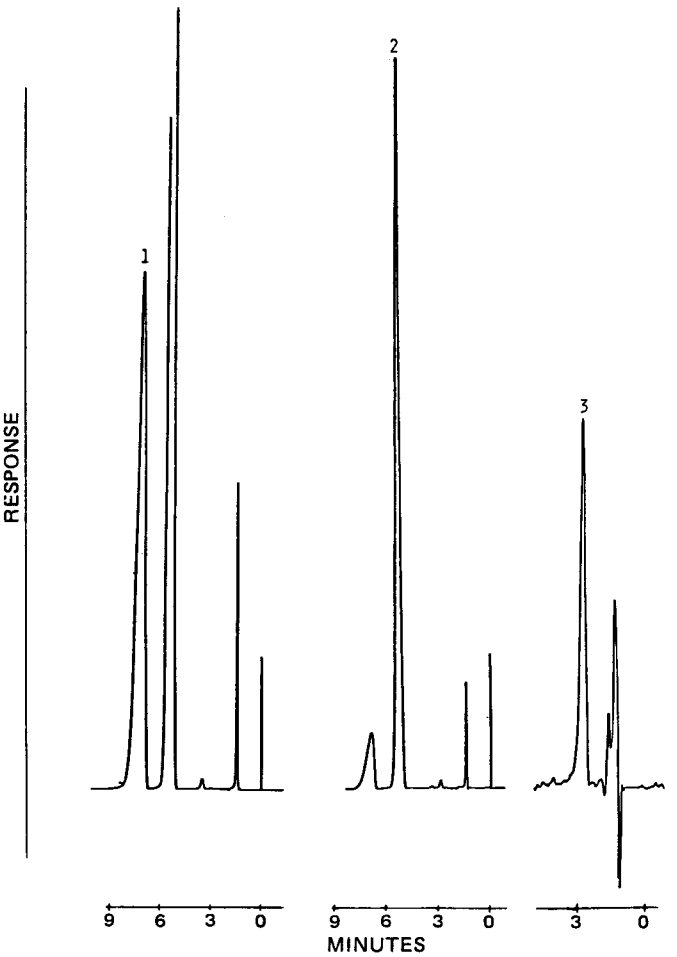

Figure 1—*Representative chromatograms of a 2-μl injection of a 2% lidocaine hydrochloride with 1:100,000 epinephrine solution, showing simultaneous detection of lidocaine (1) with UV detector at 254 nm, methylparaben (2) with UV detector at 280 nm, and epinephrine (3) with electrochemical detector at +0.90 v.*

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A high-performance liquid chromatographic (HPLC) method for epinephrine in local anesthetic solutions, which utilized a UV detector, was developed in this laboratory, but it lacked the specificity and sensitivity needed to be stability indicating. The development of electrochemical detectors for HPLC (8) and their subsequent application in the analysis of catecholamines in biological systems (9, 10) opened an entirely new possibility for the analysis of epinephrine in local anesthetic preparations. By coupling the electrochemical detector in series with a UV detector, it was possible to develop a specific, stability-indicating method that not only allowed for improved analysis of epinephrine but also permitted the simultaneous analysis of methylparaben and lidocaine hydrochloride (Fig. 1).

EXPERIMENTAL

Apparatus—Analyses were performed on a liquid chromatograph[1] equipped with a 6000-psi solvent delivery system[2] and a septumless injector[3]. Detection of methylparaben and lidocaine was carried out with a dual-channel fixed-wavelength UV detector[4]. The methylparaben was quantitated at 280 nm, and the lidocaine hydrochloride was quantitated at 254 nm. Detection of epinephrine was carried out with a glassy carbon thin-layer detector cell[5] operated with an amperometric controller[6] at a potential of +0.90 v relative to a silver–silver chloride reference electrode[7]. The inlet of the electrochemical detector was connected to the outlet of the UV detector. The prepacked column[8] (30 cm × 4 mm i.d.) was operated at ambient temperature at a flow rate of 2.0 ml/min.

Reagents—1-Octanesulfonic acid sodium salt[9], edetate disodium[10], acetic acid[11], acetonitrile[12], methanol[13], methylparaben[14], and lidocaine hydrochloride monohydrate USP[15] were used as received. Epinephrine bitartrate USP reference standard[16] was dried in a vacuum desiccator over silica gel for at least 3 hr before use and subsequently stored in a desiccator over silica gel.

Mobile Phase—The mobile phase consisted of 0.01 *M* 1-octanesulfonic acid sodium salt, 0.1 m*M* edetate disodium, 2% (v/v) acetic acid, 2% (v/v) acetonitrile, and 1% (v/v) methanol in high quality distilled water. To minimize random electrical noise spikes from the electrochemical detector, the mobile phase should be degassed daily by filtering through a 0.45-μm filter[17] connected to an aspirator.

Stock Solutions—*Epinephrine*[18]—An appropriate quantity (Table I) of epinephrine bitartrate USP reference standard was weighed accurately into the appropriate size volumetric flask, 0.01 *N* HCl was added, and the solution was diluted to volume with 0.01 *N* HCl and mixed. The solution is stable for 1 week if refrigerated.

Methylparaben—In a 250-ml volumetric flask, 625 mg of methylparaben (accurately weighed) was added to 10 ml of acetonitrile and stirred until completely dissolved. The solution was diluted to volume with distilled water and mixed. Then the solution was cooled to room temperature and diluted to volume with distilled water. Occasionally, upon standing, some methylparaben crystallized out; it was redissolved by heating, but care was taken to recool to room temperature before pipetting for preparation of the standards.

Lidocaine Hydrochloride[18]—An appropriate quantity (Table I) of lidocaine hydrochloride monohydrate USP was weighed accurately into a 100-ml volumetric flask and distilled water was added; then the solution was diluted to volume with distilled water and mixed.

Table I—Weight Guidelines for the Preparation of Stock Solutions and Standards for Various Lidocaine Hydrochloride–Epinephrine Products

Sample	Stock Solution	Recommended Dilution	Resulting Concentration
	Epinephrine[a]		
1:50,000	~18 mg/100 ml of 0.01 *N* HCl	5:25	~20 μg/ml
1:100,000	~23 mg/250 ml of 0.01 *N* HCl	5:25	~10 μg/ml
1:200,000	~23 mg/500 ml of 0.01 *N* HCl	5:25	~5.0 μg/ml
	Methylparaben[b]		
1.00 mg/ml	~625 mg/250 ml of distilled water	10:25	~1.0 mg/ml
	Lidocaine Hydrochloride[c]		
0.5%	~1.56 g/100 ml of distilled water	9:25 (8:25)	~5.3 (~4.7), mg/ml
1.0%	~3.11 g/100 ml of distilled water	9:25 (8:25)	~10.5 (~9.4), mg/ml
1.5%	~4.67 g/100 ml of distilled water	9:25 (8:25)	~15.8 (~14.1), mg/ml
2.0%	~6.22 g/100 ml of distilled water	9:25 (8:25)	~21.0 (~18.7), mg/ml

[a] Epinephrine (μg/ml) = [epinephrine bitartrate (mg)/volume (ml)] × (183.21/333.30) × (5/25) × 1000. [b] Methylparaben (mg/ml) = [methylparaben (mg)/250] × (10/25). [c] Lidocaine hydrochloride (mg/ml) = [lidocaine HCl · H_2O (g)/100] × (270.8/288.8) × [9(8)/25] × 1000.

Standard Solutions—Two standards (one at ~95% and one at ~105% of the expected lidocaine hydrochloride concentration) are required for the quantitation of the lidocaine hydrochloride if calculated utilizing peak heights. (Preliminary data indicated that only one standard is necessary if peak areas are utilized for the quantitation.)

Standard I—Five milliliters of the appropriate (Table I) epinephrine stock solution, 10 ml of methylparaben stock solution, and 9 ml of the appropriate (Table I) lidocaine hydrochloride stock solution were pipetted into a 25-ml volumetric flask, diluted to volume with distilled water, and mixed.

Standard II—Five milliliters of the epinephrine stock solution used for Standard I, 10 ml of methylparaben stock solution, and 8 ml of the lidocaine hydrochloride stock solution used for Standard I were pipetted into a 25-ml volumetric flask, diluted to volume with distilled water, and mixed.

The lidocaine hydrochloride monohydrate salt was used to prepare the stock solutions, but the final concentrations of the standards are reported as anhydrous lidocaine hydrochloride.

Chromatography—The column was conditioned with mobile phase for 0.5 hr at a flow rate of 2 ml/min before use. The electrochemical detector was turned on 0.5 hr before use. Standard I (2 μl) was injected repeatedly until the peak height response for all peaks of interest was reproducible to ±1–2%. The retention times of epinephrine, methylparaben, and lidocaine are ~3, ~5, and ~7 min, respectively. The flow rate was varied slightly to compensate for minor column-to-column differences. Two-microliter injections of the standards and samples were made, ensuring that each series of samples was bracketed by an injection of Standards I and II.

Calculations—The following calculations were used.

1. epinephrine (micrograms per milliliter) = (peak height of epinephrine in sample divided by average peak height of epinephrine in Standards I and II) × concentration of epinephrine in standards (micrograms per milliliter)

2. methylparaben (milligrams per milliliter) = (peak height of methylparaben in sample divided by average peak height of methylparaben in Standards I and II) × concentration of methylparaben in standards (milligrams per milliliter)

For lidocaine hydrochloride (milligrams per milliliter)[19], the equation of the line is determined where Y_I and Y_{II} are the peak heights for lidocaine hydrochloride in Standards I and II and X_I and X_{II} are the concentrations (milligrams per milliliter) of lidocaine hydrochloride in Standards I and II, respectively. The peak height (Y_s) for lidocaine hy-

[1] Model ALC/GPC 204, Waters Associates, Milford, MA 01757.
[2] Model 6000A, Waters Associates, Milford, MA 01757.
[3] Model U6K, Waters Associates, Milford, MA 01757.
[4] Model 440, Waters Associates, Milford, MA 01757.
[5] Model TL-5, Bioanalytical Systems, West Lafayette, IN 47906.
[6] Model LC-4, Bioanalytical Systems, West Lafayette, IN 47906.
[7] Model RC-2, Bioanalytical Systems, West Lafayette, IN 47906.
[8] μBondapak CN, 10 μm, Waters Associates, Milford, MA 01757.
[9] Eastman Kodak Co., Rochester, NY 14650.
[10] Analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, MO 63134.
[11] Analytical reagent grade, J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[12] Low UV, distilled in glass, Burdick & Jackson Laboratories, Muskegon, MI 49442.
[13] Anhydrous, distilled in glass, Burdick & Jackson Laboratories, Muskegon, MI 49442.
[14] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[15] Astra Pharmaceutical Products, Worcester, MA 01606.
[16] USP–NF Reference Standards, Rockville, MD 20852.
[17] Type HA, Millipore Corp., Bedford, MA 01730.
[18] Appropriate dilutions of the stock solutions may be made instead of separate stock solutions for each concentration.
[19] This entire calculation can be performed on any calculator capable of linear regression analysis.

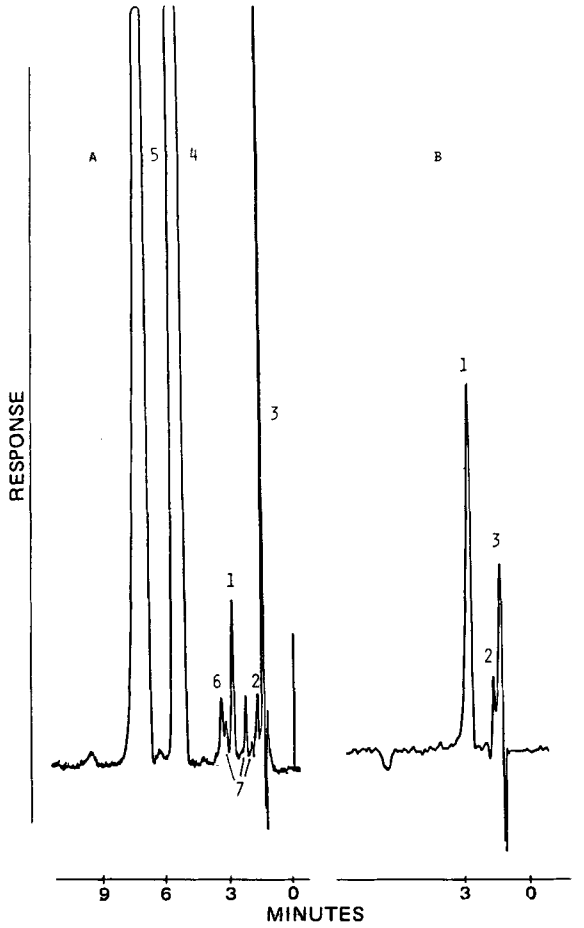

Figure 2—*(A) Representative chromatogram of a 2-μl injection of a 2% lidocaine hydrochloride with 1:100,000 epinephrine solution with UV detector at 280 nm and 0.005 aufs. Key: 1, epinephrine; 2, epinephrine sulfonic acid; 3, sodium metabisulfite; 4, methylparaben; 5, lidocaine hydrochloride; 6,* p*-hydroxybenzoic acid; and 7, package extractables and unknowns. (B) Representative chromatogram of a 2-μl injection of the same solution as in A with electrochemical detector at +0.90 v and 50 na/v. Key: 1, epinephrine; 2, epinephrine sulfonic acid; and 3, sodium metabisulfite.*

drochloride in the sample is substituted into the equation, which is solved for the concentration (X_s).

RESULTS

Precision—The precision (reproducibility), reported as the relative standard deviation, was better than ±1% for epinephrine, methylparaben, and lidocaine hydrochloride as determined on 10 replicate injections.

Linearity—The linearity of response was determined by preparing samples containing known quantities of the components of interest in a range from ~80–120% of the theoretical labeled quantity at each concentration[20]. All calibration curves were linear, with correlations coefficient >0.99.

Accuracy—Accuracy was determined by preparing simulated samples containing known quantities of the components of interest along with all excipients. The percent recoveries were determined by four different operators on two liquid chromatographs over 1 week, indicating that a day-to-day accuracy of ±1% could be obtained routinely: epinephrine, 99.9 ± 0.6% (98.1–100.9%), n = 34; methylparaben, 99.8 ± 0.5% (99.0–100.6%), n = 8; and lidocaine hydrochloride, 100.2 ± 0.9% (98.4–101.8%), n = 30.

Specificity—Simulated samples to which the potential degradation products had been added were injected, and the chromatograms were recorded. The potential degradation products were separated from the components of interest (Table II), indicating that the HPLC method is stability indicating.

Table II—HPLC Retention Values for Epinephrine, Methylparaben, Lidocaine Hydrochloride, and Their Potential Degradation Products

Compound	Retention Time, min	k'
Epinephrine sulfonic acid	1.8	0.29
Adrenochrome	2.2	0.57
Epinephrine	3.0	1.14
p-Hydroxybenzoic acid	3.2	1.29
Methylparaben	5.0	2.57
2,6-Xylidine	4.4	2.14
Lidocaine hydrochloride	6.8	3.86

Further proof of this stability-indicating capability was obtained by subjecting production batches[21] of lidocaine hydrochloride with epinephrine injection to various conditions of stress[22] and assaying for epinephrine, methylparaben, and lidocaine hydrochloride. The results indicated that the major degradation products were those that had been proposed (Table II) and also that any other unknown degradation products did not interfere with the assays.

A comparison of the chromatograms obtained from analysis of the same solution[23] with the UV and electrochemical detectors clearly showed the advantages gained in specificity and sensitivity from the electrochemical detectors. Not only the degradation products but also the package extractables interfered with the accurate quantitation of epinephrine with the UV detector (Fig. 2).

An electrochemically active peak was observed that eluted from the column immediately after epinephrine (Fig. 3). This peak was identified

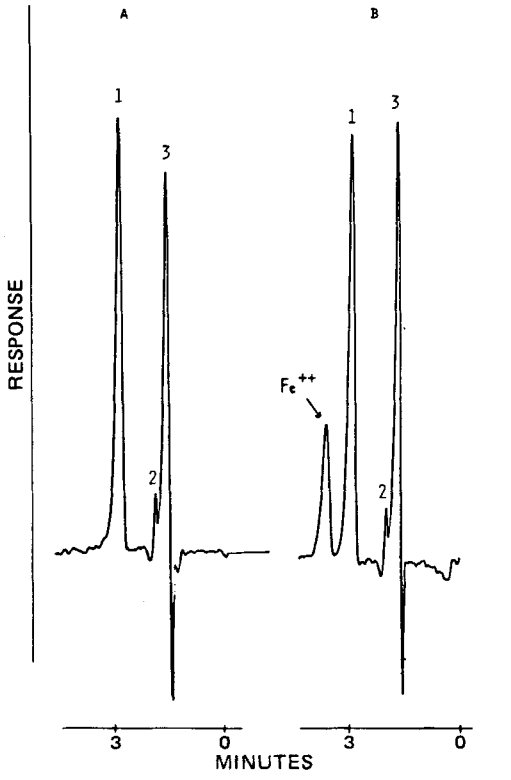

Figure 3—*Representative chromatograms of a 2-μl injection of a 2% lidocaine hydrochloride with 1:100,000 epinephrine solution. Key: A, with 0.1 m*M *disodium edetate in the mobile phase; B; without disodium edetate; 1, epinephrine; 2, epinephrine sulfonic acid; and 3, sodium metabisulfite.*

[20] Epinephrine concentrations were 1:50,000; 1:100,000; and 1:200,000. The methylparaben concentration was 1.00 mg/ml. The lidocaine hydrochloride concentrations were 0.5, 1.0, and 2.0%. The method was developed so that solutions containing 0.5–2.0% lidocaine hydrochloride could be assayed without any sample preparation. If the lidocaine hydrochloride concentration in a sample to be assayed is >2.0%, an appropriate dilution with distilled water should be made so that the lidocaine hydrochloride concentration is between 0.5 and >2.0%.

[21] Astra Pharmaceutical Products, Worcester, MA 01606.

[22] Autoclaving for 5, 10, and 20 cycles, storage for 1 month at 80 and 110°, exposure to atmospheric oxygen, addition of 12 ppm of aluminum, and an increase in the pH of the solution.

[23] Product at its expiration date.

Table III—Comparison of the HPLC Method with the USP XX or Current Methodology

Assay	Number of Batches Assayed	Percent Difference[a]
Epinephrine	115	−0.3 (USP–HPLC)
Methylparaben	87	+2.5 (UV–HPLC)
Lidocaine hydrochloride	87	−2.3 (USP–HPLC)

[a] Mean percent difference for batches assayed.

as Fe^{2+} (11) and did not interfere with the assay. However, addition of 0.1 m*M* edetate disodium to the mobile phase resulted in complete elimination of the Fe^{2+} peak with no other effect on the chromatogram.

Comparison of HPLC Method with USP or Current Methodology—Production samples[21] of lidocaine hydrochloride with epinephrine in injectable solutions, which included vials, ampuls, and dental cartridges from batches < 1 month old to batches at their expiration date, were assayed by HPLC, fluorometry (epinephrine), titrimetry (lidocaine hydrochloride), and spectrophotometry (methylparaben). All analyses were performed on individual dosage units (if enough solution was available) or on aliquots from a pool of several dosage units (if one unit was inadequate). The results (Table III) indicate good agreement between the methods.

DISCUSSION

The HPLC procedure provides a significant advance over existing methodology for assaying components in epinephrine-containing local anesthetic injectable solutions. Three of the major components (epinephrine, lidocaine hydrochloride, and methylparaben) of these types of dosage forms may be assayed simultaneously in one injection. The current methodology requires separate analytical procedures for each component.

The small sample size (2 μl) allows replicate analysis of the three components from individual dosage units. The current methodology in some cases requires units to be pooled to obtain a sufficient sample for analysis.

No preparation of the sample is necessary. The dosage unit is opened, a 2-μl aliquot is removed with a 10-μl syringe, and the sample is injected into the chromatograph. The current methodology requires extensive sample preparation for each component.

The HPLC method is selective and specific for all three components and is stability indicating.

Although both the HPLC and current methodology may be automated, the HPLC method offers a distinct advantage since only one setup of automated equipment is needed. The current methodology requires three separate and different automated setups since the procedure for each component is different. The HPLC methodology can be used to assay other local anesthetic injectable solutions such as prilocaine hydrochloride, etidocaine hydrochloride, mepivacaine hydrochloride, and bupivacaine hydrochloride with little or no change in the mobile phase.

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GC and GC–Mass Spectrometric Determination of *p*-Hydroxyphenobarbital Extracted from Plasma, Urine, and Hepatic Microsomes

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Abstract □ Analytical methodology was developed for the quantitation of *p*-hydroxyphenobarbital extracted from plasma, urine, and hepatic microsomes. *p*-Hydroxyphenobarbital was derivatized with an appropriate *n*-alkyl iodide in the presence of a methanolic base in aprotic solvent medium. The peralkylated derivatives were stable indefinitely and were quantitated by the sensitive and selective method of GC nitrogen-selective detection and/or selected ion monitoring. The accuracy, precision, and cross verification of all methods were good. The analysis was subsequently used to study the effects of other drugs on phenobarbital biodisposition.

Keyphrases □ *p*-Hydroxyphenobarbital—determination by GC and GC–mass spectrometry in urine, plasma, and hepatic microsomes □ Phenobarbital—quantitation of *p*-hydroxyphenobarbital by GC and GC–mass spectrometry in urine, plasma, and hepatic microsomes □ GC—nitrogen-selective detection of *p*-hydroxyphenobarbital in urine, plasma, and hepatic microsomes

Phenobarbital (I) is commonly used in the treatment of epilepsy, and *p*-hydroxyphenobarbital (II) is its major metabolite. Even though *p*-hydroxyphenobarbital is considered to be devoid of antiepileptic activity, its quantitation is important when studying phenobarbital biodisposition. During a study on the mechanisms of val-

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