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Research Article

A VALIDATED REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS DETERMINATION OF FIVE ANTIEPILEPTIC DRUGS USED IN THE TREATMENT OF LENNOX–GASTAUT SYNDROME IN THEIR PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: Lennox–Gastaut syndrome (LGS) is mainly treated with antiepileptic drugs (AEDs) but using one AED is not sufficient to relieve all or even most patients. A combination of agents is usually preferred. In the current study, an isocratic, selective, sensitive, precise, and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous determination of rufinamide (RUF), lamotrigine (LAM), clonazepam (CLO), valproic acid (VAL), and diazepam (DIA) which are commonly used in the management of LGS in their dosage forms using lacosamide as internal standard.

Methods: The method depends on using RESTEK C_{18} column (5 µm, 250 mm × 4.6 mm) and a mobile phase composed of acetonitrile:water (55: 45, v/v), pH = 3.3 adjusted with phosphoric acid. The method was conducted in an isocratic mode with a flow rate of 1ml/min and ultraviolet detection at 210 nm.

Results: The linearity range was 2–40 µg/ml for RUF and DIA, 0.5–40 µg/ml for LAM and CLO, and 36–180 µg/ml for VAL.

Conclusion: Statistical analysis revealed no significant difference between the results obtained and the official or reported ones for each cited drug. The method is simple to be easily implemented in quality control studies of the mentioned drugs in their pharmaceutical preparations.

Keywords: Rufinamide, Lamotrigine, Clonazepam, Valproic acid, Diazepam, High-performance liquid chromatography, Dosage form.

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INTRODUCTION

Lennox–Gastaut syndrome (LGS) is a severe form of childhood epileptic encephalopathy with multiple etiologies, whether genetic, structural, metabolic, or unknown. LGS could be diagnosed by EEG, usually demonstrating high voltage, bifrontal 1.5–2.5 Hz spikes, and wave complexes. Several seizure types are associated with LGS including sudden tonic–atonic seizures (drop attacks), atypical absence (the most common), myoclonic seizures, generalized tonic–clonic seizures, and partial onset seizures. The optimum treatment for LGS has yet to be established [1-4]. Treatment is aimed at reducing seizure burden using the least number of medications while minimizing side effects. However, seizure freedom is rare, and multiple anticonvulsants are often required. Surveys have shown that valproate is often the preferred drug for initial therapy. Either lamotrigine (LAM) or topiramate or rufinamide (RUF) is often the second-line choice for monotherapy if valproate is not efficacious. BZDs have been used as parenteral or rectal agent (diazepam [DIA], lorazepam, and midazolam) in acute cases, while for chronic oral use, clobazam, clonazepam [CLO], clorazepate, and nitrazepam were used. BZDs remain, in most guidelines, the treatment of choice for acute or subacute seizures [5-15].

On searching literature, it was found that many high-performance liquid chromatography (HPLC) methods were recently reported for the determination of RUF (Fig. 1a) [16-19], LAM (Fig. 1b) [20-23], CLO (Fig. 1c) [24,25], DIA (Fig. 1d) [26-28], and valproic acid (VAL) (Fig. 1e) [29-32] in their dosage forms. No method was reported for the simultaneous determination of the five cited drugs yet. The aim of the current work was to develop a sensitive, selective, and precise chromatographic method able to separate and quantify the cited drugs in their dosage forms. This method could also be used in the assays of the cited drugs in biological fluids as it covers their therapeutic ranges.

EXPERIMENTAL

Instrumentation

An HPLC instrument (Agilent 1100 series) was equipped with an Agilent isocratic pump G1310A, Agilent ultraviolet (UV)-visible detector G1314A, an Agilent manual injector G1328B with (20 µl) injector loop and RESTEK C_{18} column (5 µm, 4.6 × 250 mm, made in USA). An Agilent syringe (50 µl, USA) and a Powersonic 405 ultrasonic processor (Human Lab INC - Hwaseong City, Korea) were employed. The pH measurements were carried out using a pH meter (Jenway, 3505, Essex, U.K.). The mobile phase was filtered through 0.45µm nylon membrane filters (Sigma-Aldrich Co., Germany).

Materials and reagents

RUF (its purity was certified as 99.45%) and lacosamide, used as internal standard (IS) (Fig. 1f), were purchased from Wuhan Sunrise Technology Development Company, Wuhan, China. DIA, LAM, CLO, and VAL were supplied by the National Organization for Drug Control and Research, Egypt (certified to contain 99.91%, 99.98%, 99.53%, and 100.10%, respectively). Prepared Banzel® tablets were used because of its unavailability in the local market while Valium® ampoules (labelled to contain 10 mg of DIA per ampoule) were manufactured by Roche, Lamictal™ tablets (labelled to contain 25 mg of LAM per tablet, Batch No. AC0602) were manufactured by GlaxoSmithKline Pharmaceuticals, Apetryl® tablets (labelled to contain 0.5 mg of CLO per tablet) were manufactured by APEX Pharma, and Depakine® tablets (labelled to contain 200 mg of sodium valproate per tablet equivalent to 173.49 mg

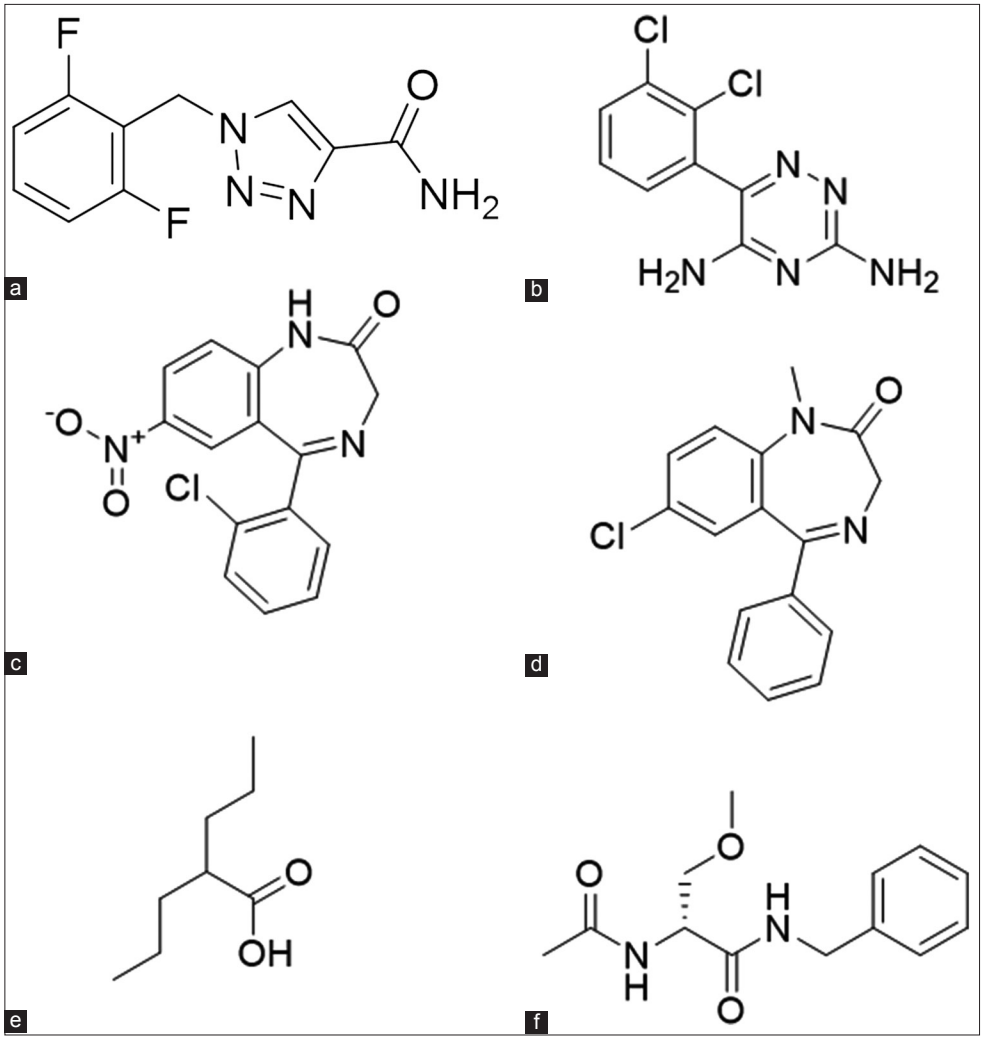

Fig. 1: Chemical structures of rufinamide (a), lamotrigine (b), clonazepam (c), diazepam (d), valproic (e), and lacosamide (IS) (f)

of VAL, Batch No. C13958) were manufactured by SANOFI and were purchased from the local market. Acetonitrile (HPLC grade) was supplied from Sigma-Aldrich, Germany. Double-distilled water was used after filtration through 0.45 µm nylon membrane filters. *o*-phosphoric acid (EL-Nasr Pharmaceutical Chemicals Co., Egypt) was prepared as 0.01 N aqueous solution.

Chromatographic conditions

Chromatographic separation was achieved on RESTEK C_{18} column (5 µm, 4.6 × 250 mm), applying isocratic elution using a mobile phase consisting of acetonitrile:water (55:45, v/v, adjusted with 0.01 N aqueous solution of *o*-phosphoric acid to pH = 3.3). The mobile phase was filtered through a membrane filter of 0.45 µm porosity and pumped through the column at a flow rate of 1 ml/min. Analysis was performed at ambient temperature, and the UV detector was set at 210 nm.

Stock and working solutions preparation

Standard stock solutions of (200 µg/ml) for RUF, LAM, CLO, DIA, and IS and also a stock solution of (9 mg/ml) for VAL were prepared in acetonitrile by transferring an accurately weighed amount of each drug in a series of 50 ml volumetric flask, adding 25 ml acetonitrile, then the mixture was sonicated and the flask was completed to volume with the same solvent. For the preparation of working solutions of 50 µg/ml for RUF, LAM, CLO, and DIA, 25 ml was transferred from the stock solution of each drug into a 100 ml volumetric flask and completed with acetonitrile to volume. Furthermore, two working solutions of 900 µg/ml and 100 µg/ml for VAL and IS, respectively, were prepared similarly.

Sample preparation

Twenty tablets of Depakine®, Apetryl®, Lamictal®, and Banzel® were separately weighed and finely powdered. A quantity of each powdered tablets equivalent to 10 mg of VAL (equivalent to 11.53 mg of sodium valproate), CLO, LAM, and RUF, respectively, was accurately weighed, 25 ml of acetonitrile was added, and each drug was extracted by sonication for 15 min. The volume was completed to 50 ml with acetonitrile; the solution was mixed well and filtered on dry funnel and dry filter paper discarding the first few milliliters to obtain a sample stock solution of 200 µg/ml. Further, dilution was carried out using acetonitrile to obtain sample working solutions of 50 µg/ml of CLO, LAM, and RUF and 90 µg/ml of VAL. Furthermore, one ampoule of valium was transferred to a 50 ml volumetric flask, and the volume was completed with acetonitrile to prepare a sample stock solution of (200 µg/ml) from which a sample working solution of 50 µg/ml of DIA was also prepared in the same manner.

General procedures and linearity

Accurately measured aliquots of RUF, DIA, LAM, and CLO standard solutions (50 µg/ml) equivalent to 20–400 µg of RUF and DIA and 5–400 µg of LAM and CLO, respectively, were transferred into a series of 10 ml volumetric flasks and completed to volume with acetonitrile. Furthermore, different aliquots were transferred from VAL standard solution (90 µg/ml) to produce solutions of the concentration range of 36–180 µg/ml. A volume of 20 µl of each solution was injected in triplicates into the chromatograph. The chromatographic conditions were adjusted as mentioned under section chromatographic conditions. The recorded AUPs × 10^{-3} were plotted versus the corresponding

concentrations of RUF, DIA, LAM, CLO, and VAL to obtain the calibration curves.

RESULTS AND DISCUSSION

Method development

The current work aimed to develop an accurate, sensitive, and precise chromatographic method to separate and simultaneously determine RUF, DIA, LAM, CLO, and VAL. A variety of mobile phases were investigated in the development of the present method where different proportions of methanol: Water and acetonitrile: Water and phosphate buffer: Acetonitrile at different pH was attempted as mobile phases, but it was found that the presence of buffer is not needed. The use of acidified water was satisfactory for the separation of the peaks, but adjusting the pH was a very important step due to the big differences in pKa of the cited drugs. Finally, a mobile phase composed of acetonitrile:water (55: 45, v/v), pH = 3.3 adjusted with phosphoric acid, was satisfactory to achieve the separation and resulted in symmetric peaks of the cited drugs with good retention times. The detection wavelength was selected to be 210 nm which is suitable for the determination of the cited drugs as it represents the maximum absorption wavelength of each drug. By adjusting all the chromatographic conditions, a good separation of RUF, DIA, LAM, CLO, and VAL using lacosamide as IS was achieved with the following retention times: 3.102, 8.365, 4.235, 4.879, 7.242, and 3.712 for IS, respectively (Fig. 2).

Method validation

The optimized chromatographic method was validated by evaluating linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, and system suitability parameters in accordance with the ICH guideline Q2 (R1) [33].

Linearity

The linearity was investigated at six concentration levels of the standard solutions of RUF, DIA, LAM, CLO, and VAL; each concentration was analyzed 3 times. The linearity was evaluated by linear regression analysis. The correlation coefficients were 0.9998 for RUF, DIA and VAL, 0.9999 for CLO, and 0.9992 for LAM, respectively. The analytical data of the calibration curves including standard deviations (SD) for the slope (S_b) and intercept (S_a), confidence limits of the slope, and intercept are summarized in Table 1.

Accuracy

The accuracy of the proposed method was tested by analyzing triplicate samples of standard solutions of each cited drug. The recovery percentages are stated in Table 2, and the results revealed the high accuracy of the proposed method. Furthermore, method accuracy was assessed as recovery obtained when spiking the sample solution with known concentrations of the cited drugs (standard addition technique) as shown in Tables 3-7.

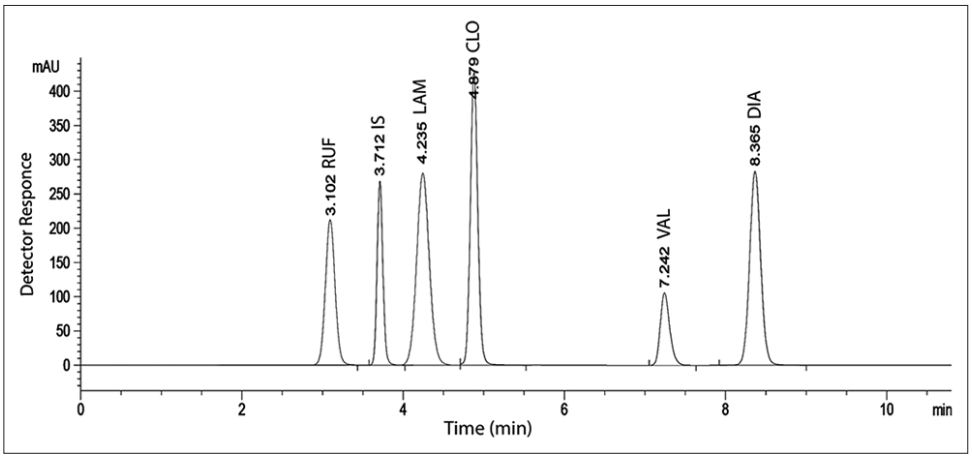

Fig. 2: High-performance liquid chromatogram of rufinamide, lamotrigine, clonazepam, diazepam, valproic acid, and lacosamide (IS) in their laboratory prepared mixture

Table 1: Validation parameters and results obtained by the proposed RP-HPLC method for the simultaneous determination of RUF, DIA, LAM, CLO, and VAL

Item	RUF	DIA	LAM	CLO	VAL
Retention time (t_R) (min)	3.102	8.365	4.235	4.879	7.242
Wavelength of detection (nm)	210	210	210	210	210
Range of linearity	2–40 µg/ml	2–40 µg/ml	0.5–40 µg/ml	0.5–40 µg/ml	36–180 µg/ml
Regression equation	PAR=0.1004 C_{RUF}−0.0781	PAR=0.1296 C_{DIA}+0.1078	PAR=0.1048 C_{LAM}+0.0880	PAR=0.1310 C_{CLO}+0.0612	PAR=0.0097 C_{VAL}+0.0172
Regression coefficient (r^2)	0.9998	0.9998	0.9992	0.9999	0.9998
LOD (µg/ml)	0.5263	0.4226	0.1362	0.1258	5.4411
LOQ (µg/ml)	1.5948	1.2807	0.4127	0.3813	16.4881
SD of the slope (Sb)	0.0007	0.0009	0.0015	0.0007	0.0001
Standard deviation of the intercept (Sa)	0.0160	0.0199	0.0340	0.0159	0.0094
Confidence limit of the slope	0.1004±0.0020	0.1296±0.0025	0.1048±0.0042	0.1310±0.0020	0.0097±0.0002
Confidence limit of the intercept	−0.0781±0.0444	0.1078±0.0552	0.0880±0.0946	0.0612±0.0443	0.0172±0.0262
Standard error of estimation	0.0238	0.0296	0.0538	0.0252	0.0095
*Intraday % RSD	0.136–0.600	0.120–0.372	0.060–0.255	0.252–0.483	0.144–0.974
*Interday % RSD	0.153–0.456	0.084–0.774	0.129–0.779	0.431–0.658	0.166–0.312

*PAR: Peak area ratio, **LOD: 3.3*SD/slope, ***LOQ: 10*SD/slope. ****The intraday (n=3), average of three concentrations of 3, 15, and 35 µg/ml for RUF and DIA, 1.5, 15, and 35 µg/ml for LAM and CLO, and 45, 135, and 171 µg/ml for VAL repeated 3 times within the day. *****The interday (n=3), average of three concentrations of 3, 15, and 35 µg/ml for RUF and DIA, 1.5, 15, and 35 µg/ml for LAM and CLO, and 45, 135, and 171 µg/ml for VAL repeated 3 times in 3 successive days. RP-HPLC: Reversed-phase high-performance liquid chromatography, RUF: Rufinamide, LAM: Lamotrigine, CLO: Clonazepam, DIA: Diazepam, VAL: Valproic acid, LOD: Limit of detection, LOQ: Limit of quantification, SD: Standard deviations

Table 2: Determination of RUF, DIA, LAM, CLO, and VAL in drug substance using the proposed RP-HPLC method

Taken (µg/ml)	PAR		Taken (µg/ml)	PAR		Taken (µg/ml)	PAR	Found (µg/ml)					Recovery %				
	RUF	DIA		LAM	CLO		VAL	RUF	DIA	LAM	CLO	VAL	RUF	DIA	LAM	CLO	VAL
4	0.322	0.623	1	0.192	0.191	54	0.541	3.99	3.98	0.99	0.99	54.00	99.63	99.38	99.20	99.10	100.00
6	0.525	0.879	4	0.512	0.583	81	0.802	6.01	5.95	4.05	3.98	80.91	100.12	99.18	101.15	99.58	99.89
8	0.718	1.131	12	1.351	1.623	108	1.058	7.93	7.90	12.05	11.92	107.30	99.11	98.69	100.43	99.35	99.35
16	1.527	2.179	18	1.983	2.410	117	1.149	15.99	15.98	18.08	17.93	116.68	99.92	99.88	100.46	99.61	99.73
24	2.317	3.210	24	2.587	3.202	126	1.236	23.86	23.94	23.85	23.98	125.65	99.40	99.74	99.35	99.90	99.72
32	3.113	4.238	32	3.421	4.234	153	1.487	31.78	31.87	31.80	31.85	151.53	99.33	99.59	99.38	99.54	99.04
												Mean	99.59	99.41	100.00	99.51	99.62
												±SD	0.381	0.432	0.796	0.269	0.360
												±RSD	0.382	0.435	0.796	0.270	0.362

RP-HPLC: Reversed-phase high-performance liquid chromatography, RUF: Rufinamide, LAM: Lamotrigine, CLO: Clonazepam, DIA: Diazepam, VAL: Valproic acid, SD: Standard deviations

Table 3: Determination of RUF in Banzel® tablets applying standard addition technique using the proposed RP-HPLC method

Taken (µg/ml)		PAR		Found (µg/ml)			Recovery %	
Tablet	Added	Tablet	Tablet and added	Tablet	Tablet and added	Added	Tablet	Added
5	5	0.425	0.929	5.01	10.03	5.02	100.22	100.40
5	6	0.425	1.030	5.01	11.04	6.03	100.22	100.43
10	8	0.924	1.736	9.98	18.07	8.09	99.81	101.10
10	12	0.924	2.142	9.98	22.11	12.13	99.81	101.10
20	16	1.943	3.569	20.13	36.33	16.20	100.65	101.23
20	20	1.943	3.956	20.13	40.18	20.05	100.65	100.25
						Mean	100.23	100.75
						±SD	0.420	0.436
						±RSD	0.419	0.433

RP-HPLC: Reversed-phase high-performance liquid chromatography, RUF: Rufinamide, SD: Standard deviations

Table 4: Determination of DIA in Valium® ampoules applying standard addition technique using the proposed RP-HPLC method

Taken (µg/ml)		PAR		Found (µg/ml)			Recovery %	
Ampoule	Added	Ampoule	Ampoule and added	Ampoule	Ampoule and added	Added	Ampoule	Added
5	5	0.750	1.397	4.96	9.948	4.99	99.10	99.86
5	6	0.750	1.531	4.96	10.981	6.03	99.10	100.43
10	8	1.396	2.444	9.94	18.026	8.09	99.40	101.08
10	12	1.396	2.963	9.94	22.031	12.09	99.40	100.76
20	16	2.693	4.781	19.95	36.059	16.11	99.74	100.69
20	20	2.693	5.308	19.95	40.125	20.18	99.74	100.89
						Mean	99.41	100.62
						±SD	0.320	0.430
						±RSD	0.322	0.427

RP-HPLC: Reversed-phase high-performance liquid chromatography, DIA: Diazepam, SD: Standard deviations

Table 5: Determination of LAM in Lamictal® tablets applying standard addition technique using the proposed RP-HPLC method

Taken (µg/ml)		PAR		Found (µg/ml)			Recovery %	
Tablet	Added	Tablet	Tablet and added	Tablet	Tablet and added	Added	Tablet	Added
2	1.6	0.297	0.464	1.99	3.59	1.59	99.70	99.63
2	2	0.297	0.508	1.99	4.01	2.01	99.70	100.70
5	5	0.605	1.133	4.93	9.97	5.04	98.66	100.82
5	6	0.605	1.234	4.93	10.94	6.01	98.66	100.08
10	8	1.117	1.956	9.82	17.82	8.01	98.19	100.06
10	12	1.117	2.358	9.82	21.66	11.84	98.19	98.68
						Mean	98.85	100.00
						±SD	0.773	0.781
						±RSD	0.782	0.781

RP-HPLC: Reversed-phase high-performance liquid chromatography, LAM: Lamotrigine, SD: Standard deviations

Precision

The precision of the developed method was checked by analyzing three different concentrations of the cited drugs in triplicate during the same day (intraday precision) and on 3 consecutive days (interday precision). The results are presented in Table 1.

Specificity

Specificity was established by analyzing the cited drugs in laboratory prepared mixtures containing different ratios of the cited drugs. Specificity was also checked by analyzing each drug in its dosage form separately showing no interference from excipients (Fig. 3). The use

Table 6: Determination of CLO in Apetryl® tablets applying standard addition technique using the proposed RP-PLC method

Taken (µg/ml)		PAR		Found (µg/ml)			Recovery %	
Tablet	Added	Tablet	Tablet and added	Tablet	Tablet and added	Added	Tablet	Added
2	1.6	0.321	0.530	1.98	3.58	1.60	99.15	99.75
2	2	0.321	0.582	1.98	3.98	1.99	99.15	99.65
5	5	0.715	1.370	4.99	9.99	5.00	99.82	100.02
5	6	0.715	1.495	4.99	10.95	5.96	99.82	99.25
10	8	1.360	2.397	9.92	17.83	7.92	99.15	98.95
10	12	1.360	2.928	9.92	21.88	11.97	99.15	99.74
						Mean	99.37	99.56
						±SD	0.387	0.389
						±RSD	0.389	0.391

RP-HPLC: Reversed-phase high-performance liquid chromatography, CLO: Clonazepam, SD: Standard deviations

Table 7: Determination of VAL in Depakine® tablets applying standard addition technique using the proposed RP-HPLC method

Taken (µg/ml)		PAR		Found (µg/ml)			Recovery %	
Tablet*	Added	Tablet	Tablet and added	Tablet	Tablet and added	Added	Tablet	Added
36	36	0.366	0.713	35.96	71.73	35.77	99.89	99.37
36	45	0.366	0.801	35.96	80.80	44.85	99.89	99.66
72	54	0.712	1.229	71.63	124.93	53.30	99.48	98.70
72	72	0.712	1.398	71.63	142.35	70.72	99.48	98.23
90	72	0.884	1.576	89.36	160.70	71.34	99.29	99.08
90	90	0.884	1.746	89.36	178.23	88.87	99.29	98.74
						Mean	99.55	98.96
						±SD	0.307	0.514
						±RSD	0.308	0.519

*The concentrations mentioned above of sodium valproate are expressed in its equivalence of VAL. RP-HPLC: Reversed-phase high-performance liquid chromatography, VAL: Valproic, SD: Standard deviations

Table 8: Determination of RUF, DIA, LAM, CLO, and VAL in laboratory prepared mixtures using the proposed RP-HPLC method

Taken (µg/ml)	PAR		Taken (µg/ml)	PAR		Taken (µg/ml)	PAR	Found (µg/ml)					Recovery %				
	RUF	DIA		LAM	CLO		VAL	RUF	DIA	LAM	CLO	VAL	RUF	DIA	LAM	CLO	VAL
2	0.121	0.362	0.5	0.140	0.127	36	0.361	1.98	1.96	0.50	0.50	35.44	99.15	98.05	99.20	100.40	98.45
5	0.417	0.752	2	0.297	0.321	72	0.711	4.93	4.97	1.99	1.98	71.53	98.62	99.42	99.70	99.15	99.34
10	0.916	1.381	10	1.117	1.361	90	0.891	9.90	9.82	9.82	9.92	90.08	99.01	98.24	98.19	99.22	100.09
20	1.911	2.690	20	2.149	2.694	144	1.408	19.81	19.92	19.67	20.10	143.38	99.06	99.62	98.33	100.49	99.57
30	2.887	3.972	30	3.232	4.004	162	1.581	29.53	29.82	30.00	30.10	161.22	98.44	99.39	100.00	100.33	99.52
40	3.881	5.210	40	4.198	5.305	180	1.742	39.43	39.37	39.22	40.03	177.81	98.58	98.42	98.05	100.07	98.79
												Mean	98.81	98.86	98.91	99.94	99.29
												±SD	0.298	0.694	0.836	0.604	0.588
												±RSD	0.302	0.702	0.845	0.605	0.592

SD: Standard deviation, RP-HPLC: Reversed-phase high-performance liquid chromatography, RUF: Rufinamide, LAM: Lamotrigine, CLO: Clonazepam, DIA: Diazepam, VAL: Valproic

of phosphoric acid in the mobile phase liberates VAL from sodium valproate in its dosage form which results in a peak with the same retention time as VAL in drug substance (Fig. 3). The good recovery % and low SD proved the high specificity of the proposed method (Table 8).

LOD and LOQ

According to the ICH recommendations [33], the parameters LOD and LOQ were determined on the basis of SD of the response and slope of the regression equation, Table 1.

System suitability

The system suitability parameters with respect to the number of theoretical plates, resolution factor, tailing factor, capacity factor, and selectivity factor were displayed in Table 9.

Statistics

The proposed analytical method was compared with the reference methods of the cited drugs [19] using statistical analysis. The Student's t-test and F-test were applied and revealed no significant difference between the experimental values obtained in the pure sample analysis by the newly developed method and that of the references methods (Table 10).

CONCLUSION

The proposed RP-HPLC method was accurate, precise, selective, and sensitive. It allows the simultaneous separation and determination of five anti-epileptic drugs: RUF, DIA, LAM, CLO, and VAL in their pharmaceutical dosage forms using lacosamide as IS. The validation of the developed method according to the ICH guidelines proved the applicability and great value of this method for routine analysis in quality control laboratories for the determination of the cited drugs in their pure form and their dosage forms.

CONFLICT OF INTEREST

All of the authors declare that they have no conflict of interest.

Table 9: System suitability tests of the proposed RP-HPLC method for the simultaneous determination of RUF, DIA, LAM, CLO, and VAL

Parameter	RUF	IS	LAM	CLO	VAL	DIA	Reference value
N	14573	13315	12893	13541	19220	18288	The higher the value, the more efficient the column is
R		3.02 3.37		3.68 12.53		4.92	>2
T	0.95	0.87	0.94	0.90	0.77	0.94	≤2
K′	2.102	2.712	3.235	3.879	6.242	7.365	1–10
α		1.20 1.14		1.15 1.48		1.16	≥1

N: Number of theoretical plates, R: Resolution factor, T: Tailing factor, K′: Capacity factor, α: Selectivity factor. RP-HPLC: Reversed-phase high-performance liquid chromatography, RUF: Rufinamide, LAM: Lamotrigine, CLO: Clonazepam, DIA: Diazepam, VAL: Valproic

Table 10: Statistical comparison between the proposed reversed-phase high-performance liquid chromatography method for the simultaneous determination of RUF, DIA, LAM, CLO, and VAL in drug substance and the reference methods

Statistical term	Reference method for RUF[1]	RUF by RP-HPLC method	Reference method for DIA[2]	DIA by RP-HPLC method	Reference method for LAM[3]	LAM by RP-HPLC method	Reference method for CLO[4]	CLO by RP-HPLC method	Reference method for VAL[5]	VAL by RP-HPLC method
Mean	99.45	99.59	99.91	99.41	99.98	100.00	99.53	99.51	100.10	99.62
±SD	0.779	0.381	0.936	0.432	0.927	0.796	0.356	0.269	0.744	0.360
±SE	0.318	0.155	0.382	0.176	0.378	0.325	0.145	0.110	0.304	0.147
% RSD	0.784	0.382	0.937	0.435	0.927	0.796	0.358	0.270	0.743	0.362
n	6	6	6	6	6	6	6	6	6	6
v	0.608	0.145	0.876	0.187	0.859	0.633	0.127	0.072	0.553	0.130
t (*2.23)		0.40		1.19		0.68		0.11		1.42
F (*5.05)		4.19		4.69		1.36		1.75		4.26

*Figures in parentheses are the theoretical t and F values at p=0.05. [1]HPLC method [19]. [2]B.P. British Pharmacopoeia 2016: Non-aqueous titration. [3]B.P. British Pharmacopoeia 2016: Non-aqueous titration. [4]B.P. British Pharmacopoeia 2016: Non-aqueous titration. [5]B.P. British Pharmacopoeia 2016: Non-aqueous titration, RP-HPLC: Reversed-phase high-performance liquid chromatography, RUF: Rufinamide, LAM: Lamotrigine, CLO: Clonazepam, DIA: Diazepam, VAL: Valproic

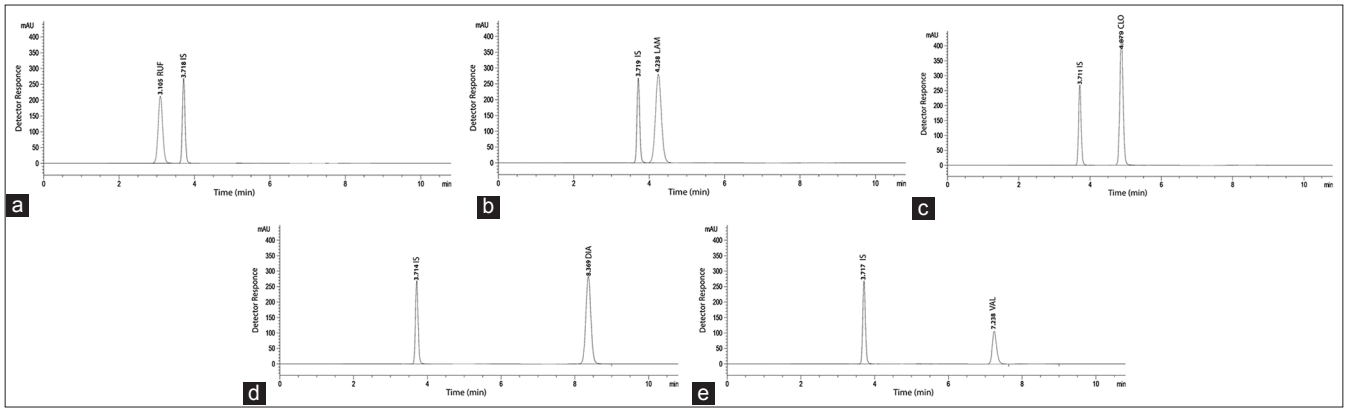

Fig. 3: High-performance liquid chromatography of rufinamide (a), lamotrigine (b), clonazepam (c), diazepam (d), and valproic (e) in their dosage forms

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