Original Research Article

Study on the quality control analysis of antiepileptic drugs using high-performance liquid chromatography

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Abstract

Background: Epilepsy is the second most common neurological condition, and antiepileptic drugs (AEDs) are used as a prophylactic measure. Any amount of poor-quality medicine is unacceptable because it increases morbidity and mortality, thus an assessment of quality of AEDs are important in developing countries like India. Phenytoin (PHT) has become a major first-line AED in the treatment of partial and secondarily generalized seizures, but like other pharmaceuticals, PHT too may develop impurities at various stages of development, transportation, and storage which make the pharmaceutical risky. Hence, the objective of the study was to test PHT concentrations in various injectable medicines available in the local markets of Kashmir Valley.

Materials and Methods: Quality analysis of PHT by high-performance liquid chromatography (HPLC) is more precise and accurate than using other analytical methods such as enzyme-based assays and immunoassay as the use of HPLC technique in the analytical field helps in structure elucidation and quantitative determination of impurities. In this study, we studied the different parameters of quality of various PHT drugs available in the local markets using HPLC and compared the results obtained with that of the standard.

Results: We observed that all the tested PHT sodium injections available in the local market have standard concentrations as all the samples under study showed the peak exactly where the standard PHT peak was supposed to be. Hence, our results suggest that the quality of various PHT drugs used in Kashmir Valley is satisfactory and safe.

Conclusion: It is clear from our results that there is a need for a quick and effective drug quality analysis method. As shown from the experiments performed in this study, HPLC is not only a suitable but also efficient system to carry out drug quality evaluation and help curb or at least keep in check the rampant sale of substandard drugs that go unabated in developing countries like India.

Keywords: Antiepileptic drugs, epilepsy, high-performance liquid chromatography, phenytoin, prophylactic, seizures

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INTRODUCTION

Epilepsy, or the "falling sickness," has a much older history than any of the other individual nervous or mental disorders. Epilepsy is the second most common neurological condition after headache and it affects some 65 million people around the world of which about

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6–10 million are found in India alone. For most people with epilepsy, the treatment for seizures includes antiepileptic drugs (AEDs). AEDs do not cure epilepsy or treat it but they are used as a prophylactic measure. It is a matter of concern that despite such a high number of epileptic patients, the diagnosis and management of epilepsy is often suboptimal in our country. To enhance the quality of life of the epileptic people living in India and to achieve optimal seizure control, appropriate AED use, along with monitoring of adverse effects, and assessment of the quality of AEDs are important. Phenytoin (PHT), an effective drug for the treatment of epilepsy, has become a major first-line AED in the treatment of partial and secondarily generalized seizures, especially in developing countries like India. Seizure disorders most often are treated with pharmacotherapy. Optimal AED therapy may completely control seizures in 60%–95% of patients.[1] Although the cost of AED treatments is relatively modest compared with new treatments for other neurologic conditions, the high prevalence and chronicity of epilepsy in developing countries like India, mean that, in public health terms, the overall cost of its treatment is high.[2] Around 90% of the people with epilepsy in developing countries are not receiving appropriate treatment due to cultural attitude, lack of prioritization, poor health system infrastructure, and inadequate supply of AEDs.[3] Since its discovery as an effective AED in 1940, PHT has become a major first-line AED in the treatment of partial and secondarily generalized seizure. Given its cost effectiveness and easy availability, PHT is the most commonly prescribed AED in developing countries like India. However, like any other pharmaceutical drugs, this AED would only serve its purpose in preventing seizures among epileptic patients if it was free from any impurities and of standard quality.[4]

Quality control is an essential operation of the pharmaceutical industry. Drugs must be marketed as safe and therapeutically active formulations whose performance is consistent and predictable. By dictionary definition, "quality control" means checking and directing the degree or grade of excellence of processes and products. It is the purpose of these operations to produce medications of superior efficacy, safety, and elegance and to provide assurance to the physician, the pharmacist, and the consumer that a given product performs uniformly and in a manner satisfactory for the purpose for which it is recommended.[5]

Pharmaceutical analysis in drug development mainly focuses on methods to identify and quantify potential new drug candidates, determine purity, identify by-products and degradation products in compatibility and stability studies, and to determine the drug substance's fate in the organism. Challenging tasks such as these require sophisticated techniques, dedicated equipment, and methods operated by highly skilled staff. In recent times, liquid chromatography, mainly high-performance liquid chromatography (HPLC), has been established as the key analytical technique not only in drug development but also in the routine quality control laboratory.[6]

The most used AED by general physicians, PHT has the molecular formula $C_{15}H_{12}N_2O_2$ and the chemical name 5,5-diphenylimidazolidine-2,4-dione with a molecular weight of 252.268 g mol-1 [Figure 1].[7] PHT alters brain cell sodium channels, which has the effect of limiting rapid firing of the brain cells. However, this AED is not free from side effects. Common side effects are unsteadiness and moderate cognitive problems. There are long-term potential cosmetic (body/face hair growth and skin problems) and bone problems (osteoporosis). Typical adult dose is 300–400 mg/day, usually with 100 mg pills. PHT can be started quickly in an emergency with intravenous administration, or a large dose of capsules if an immediate effect is required.[8]

PHT sodium (PS) has tendency to convert to its base form; PHT base during manufacturing, packaging, shelf life and in-use conditions can influence its clinical performance.[9] Small changes in PHT dose can cause large changes in serum drug levels which can lead to several grave side effects. The objective of the present work was to develop a quick and easy analytical method for quantification of PHT in various drug products available in the local market. Quality analysis of PHT by HPLC is more precise and accurate than using other analytical methods such as enzyme-based assays and immunoassay (EMIT assay) as the use of HPLC technique in the analytical field helps in structure elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulations.

MATERIALS AND METHODS

The certified PS, working standard was provided by the Government Medical College, Srinagar, as was acetonitrile (ACN) (HPLC Grade) and HPLC-grade water.

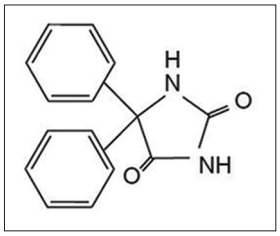

Figure 1: Chemical structure of phenytoin

Five-point calibration curve was prepared using standard PHT dissolved in 1:1 ratio of ACN and water. Five different concentrations ranging from 10 μg/ml to 100 μg/ml were prepared from stock solution (1 mg/ml) of PHT. The concentration of various samples of intravenous PHT injections available in the local market was determined using linear regression equation and was compared with their printed labels.

The HPLC system used was an Agilent 1260 Series HPLC System with a manual injector. The column used was an Eclipse XDB-C18, 4.6 mm × 150 mm, 5 μm. A mobile phase comprising ACN: water in the ratio of 1:1. The flow rate was set at 1 ml/min, the temperature of the column was maintained at 25°C, and a detection wavelength was 200 nm. Total chromatographic analysis time per sample was 6 min with PHT eluting with the retention time of 4.1 ± 0.2 min.[10]

Testing the phenytoin concentrations in various injectable medicines available in the market

For the estimation of active pharmaceutical ingredient (PHT) in various pharmaceutical formulations, a calibration curve was prepared under optimized chromatographic condition [Figure 2]. The retention time of standard PHT (100 μg/ml) is 4.06 min [Figure 3]. The average peak area of different standard preparations (10-100 μg/ml) is shown in Table 1. The PHT injections from two pharmaceutical companies were obtained from various medical shops through prescriptions and tested for their quality. The first PHT injections that were tested were from Abbott Pharmaceuticals. Established in 1910, Abbott in India is one of the country's oldest and most admired health-care companies. The injection available in the market was for 50 ml. Appropriate dilutions (20 and 100 μg/ml) were prepared from the injectable and injected into HPLC machine for quantitative determination of PHT. Each sample was injected twice to eliminate any chances of errors.

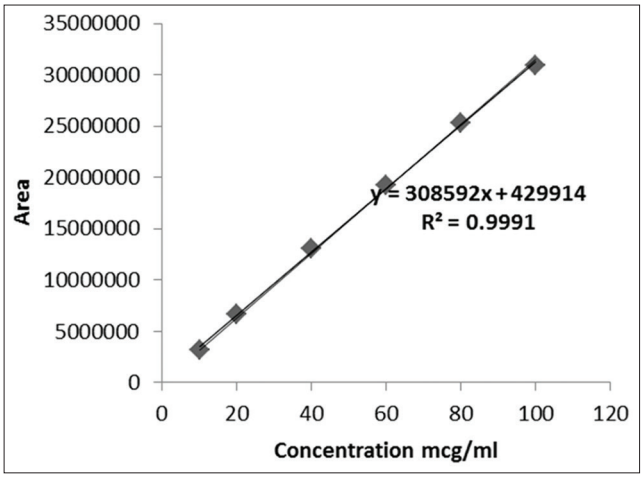

Figure 2: Phenytoin calibration curve

After obtaining the chromatograms of 20 μg/ml [Figure 4], the area under the peak and the retention time for the Abbott PHT samples [Table 2] was calculated and compared with the results of the standard used. Similarly, chromatogram of 100 μg/ml was acquired under identical conditions [Figure 5], and the area under the peak and the retention time was calculated [Table 3] and compared with the results of the standard used. The average area of all the samples injected of two selected concentrations (20 and 100 μg/ml) is shown in Table 4.

Another sample that was obtained and tested was from Bharat Pharmaceuticals which is a local Indian drug company which supplies drugs to various hospitals of Kashmir valley. This injection too is available as 50 ml, so appropriate dilutions were made in a similar way as for

Table 1: The retention time and area under the peak for various phenytoin concentrations of the standard sample

Concentration (μg/ml)	Area under peak (mAU)	Retention time (min)
10	3,138,999	4.067
20	6,632,229	4.087
40	13,045,624	4.093
60	19,206,177	4.107
80	25,353,541	4.113
100	30,866,423	4.127

Table 2: The average retention time and the average area under the peak for a concentration of 20 μg of test sample from Abbott Pharmaceuticals

For 20 μg	
Retention time (min)	Area under peak (mAU)
4.113	41,919,282
4.080	44,665,801
Average: 4.096	Average: 43,292,541.5

Table 3: The average retention time and the average area under the peak for a concentration of 100 ug of test sample from Abbott Pharmaceuticals

100 μg	
Retention time (min)	Area under peak (mAU)
4.080	33,860,813
4.073	33,261,154
Average: 4.076	Average: 33,560,983.5

Table 4: The average retention time and the average area under the peak for all concentrations of test sample from Abbott Pharmaceuticals

Abbott Pharmaceuticals	20 μg	100 μg
Retention time (min)	4.096	4.076
Area under peak (mAU)	43,292,541.5	33,560,983.5

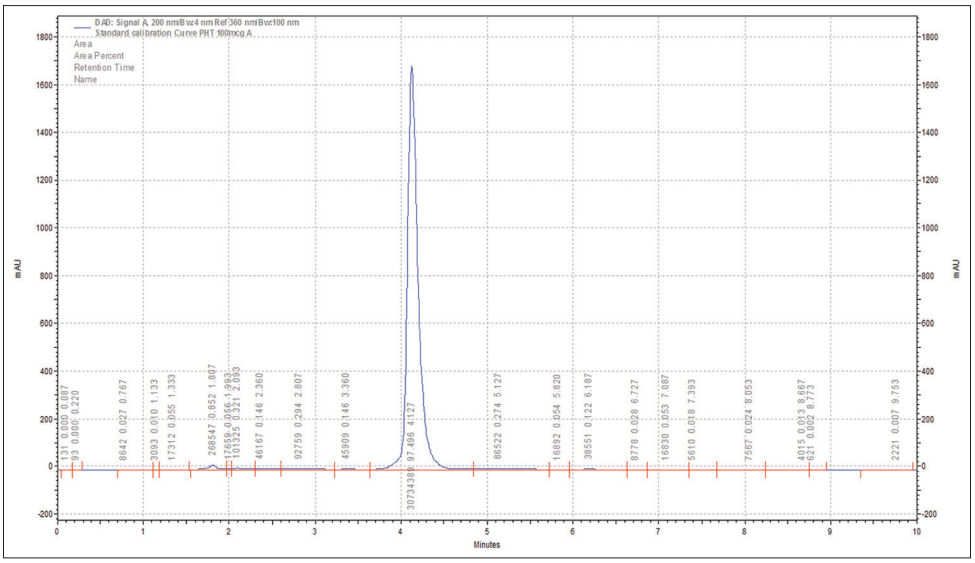

Figure 3: Chromatogram for 100 µg of standard sample of phenytoin (the peak shows the retention time)

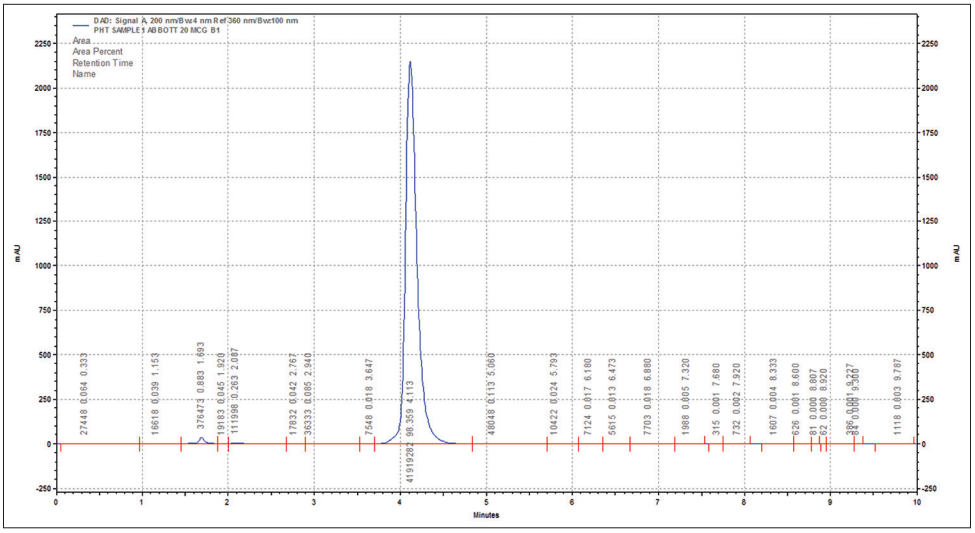

Figure 4: Chromatogram for 20 µg/ml of test sample 1 of phenytoin from Abbott Pharmaceuticals

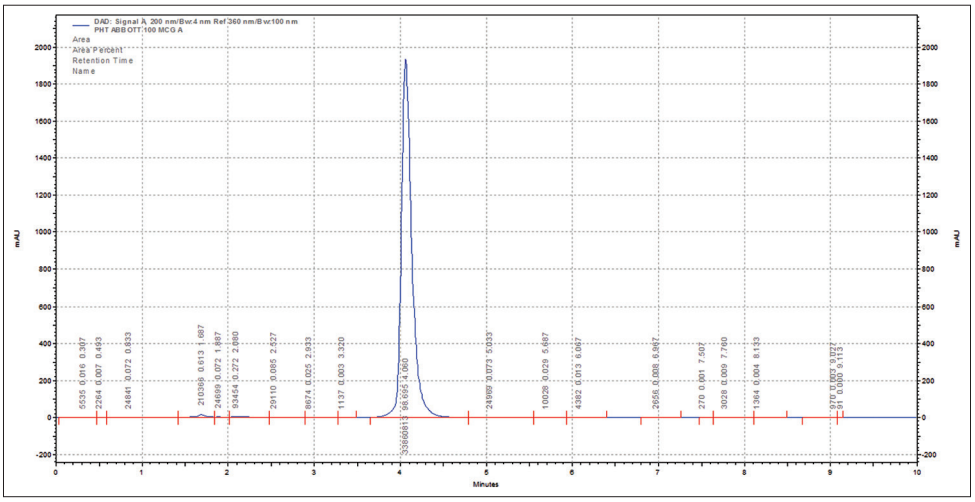

Figure 5: Chromatogram for 100 µg/ml of test sample 1 of phenytoin from Abbott Pharmaceuticals

the Abbott Pharmaceutical samples. After obtaining the chromatograms, the retention time and the peak area were calculated and compared with the result from the standard used. Similarly, dilutions were prepared for the second sample from Bharat Pharmaceuticals. Again 20 [Figure 6] and 100 μg/ml [Figure 7] samples were injected. The average area of all the samples injected of two selected concentrations (20 and 100 μg/ml) is shown in Table 5.

RESULTS

Both of the tested PHT sodium injections available in the local market have standard concentrations. Although there are slight variations between the standard and the studied samples [Table 6], the errors can be ignored as they may have risen from pipetting errors and other errors caused while handling and preparing the samples since all the samples under study showed the peak exactly where the standard PHT peak is supposed to be, i.e., near about 4.0–4.2.

DISCUSSION

There is always an incentive to increase productivity in the pharmaceutical industry, particularly in an era of increasing patent expirations, high failure rates for new chemical entity development, and high-profile drug recalls. The regulatory environment, which requires increasingly

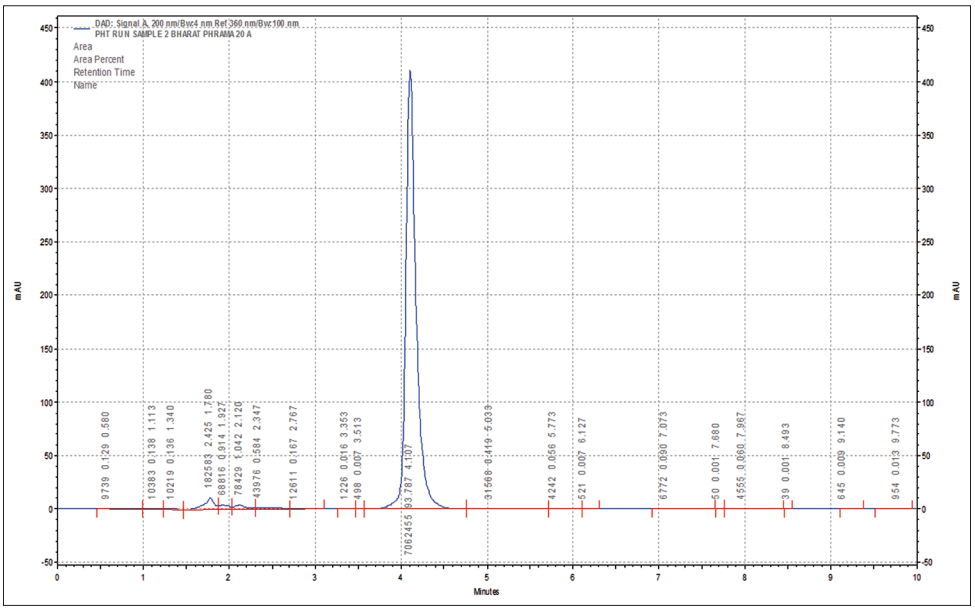

Figure 6: Chromatogram for 20 μg/ml of test sample 1 from Bharat Pharmaceuticals

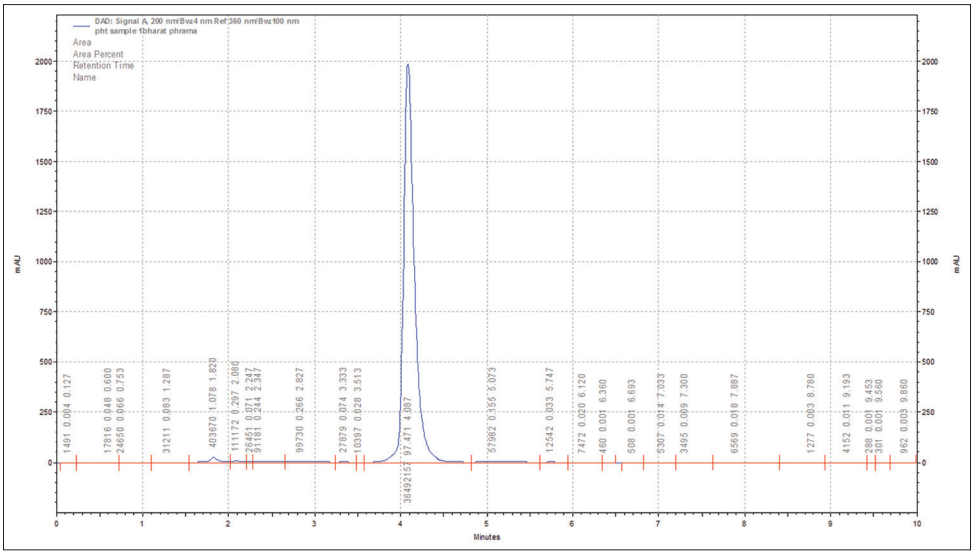

Figure 7: Chromatogram for 100 μg/ml of test sample 1 from Bharat Pharmaceuticals

Table 5: The average retention time and the average area under the peak for all concentrations of test samples from Bharat Pharmaceuticals

Bharat pharmaceuticals	20 µg	100 µg
Retention time (min)	4.117	4.086
Area under peak (mAU)	6,433,547	37,626,083

Table 6: Comparison of the average retention time and the average area under the peak for all concentrations of both the test samples with the standard

	Bharat	Abbott	Standard
Average retention time			
20 µg	4.117	4.096	4.093
100 µg	4.086	4.076	4.127
Area under peak (mAU)			
20 µg	6,433,547	43,292,541	6,632,229
100 µg	37,626,083	33,560,983	30,866,423

higher sensitivity, accuracy, and precision in quality measurements, is also challenging the industry to improve its processes. Purification, purity analysis, and impurity analysis are three areas that are vital for assuring future success in drug discovery and development.[11] India is home to over 135 Food and Drug Administration-approved pharmaceutical manufacturing units and in 2012, its INR 1.1 trillion – largely generics making – drug industry exported around INR 400 billion worth of drugs across the globe. Although the industry is growing at double-digit rates, there have been several incidents in the recent years that have created an atmosphere of mistrust among various stakeholders, particularly regarding drug quality. A press report noted that, despite regulations being introduced in 2003 to curb the import of substandard raw materials, custom authorities seized several consignments of substandard materials imported by traders.[12]

Establishing and evaluating the purity of compounds is essential across all of the stages of drug discovery and development. Key to the improvement and maintenance of drug quality is the implementation of strong regulatory control. There is a need for pharmacovigilance programs to be in place to monitor the safety of marketed drugs constantly and to communicate any safety issues to manufacturers, health-care providers, and patients. Although effective drugs are now available for some of the most prevalent and destructive diseases in the developing world, including tuberculosis, malaria, and HIV/AIDS, the effectiveness of drugs in treating these diseases, as well as many other illnesses, is compromised by the distribution of substandard drugs. Both branded and generic drugs are affected. Generic formulations offer low-cost options for many drugs, and generic substitution may be mandatory in some countries, but the quality of these drugs must be regulated. There are many levels in the drug production and marketing processes that may be influenced by corruption and lead to substandard drugs entering the market. This may occur, for example, during the construction or equipping of manufacturing facilities, during drug registration or certification, during quality-control checks, including drug testing and site inspections, and during drug procurement. One such case was highlighted when the FDA uncovered evidence that a facility in India owned by Ranbaxy Laboratories had falsified data and test results in approved and pending drug applications on at least two occasions.

It has been reported that the prevalence of poor quality drugs in the Indian market is as high as 30% while the National Drug Regulator – Central Drugs Standard Control Organization (CDSCO) has argued that such reports are unverified and not backed with sound evidence, it subsequently conducted an independent study in 2009 and reported a prevalence figure of 0.3% for the spurious drugs in the domestic market. In India, Drug Testing Laboratories comprise eight central government laboratories. However, not all of the laboratories are fully equipped. The present drug testing capacity of the laboratories is around 8000 samples per annum, which is targeted to be increased to 24,000 samples per annum. This too may be insufficient to test all products given that at present, the most conservative estimate of total pharmaceutical products in the domestic market stands at a figure of 62,000 products. At present, the Pharmacovigilance Programme of India has limited outreach among patients as well as health-care professionals, with little or no information provided about the measures taken or recommended after an Adverse Drug Report is reported. In addition, this system is not integrated with the drug alerts that are generated through random sampling at both the state and central levels.

To secure their health, consumers who have no means for verifying the authenticity or potency of drugs need to be assured at all times that medicines made available to them are of good quality and safe to use. It thus falls upon other participants at various nodes of the supply chain to provide the much-needed assurance. One of the major obstacles that both the industry and regulators face is related to the definition and interpretation of quality standards of the manufacturing process. On the one hand, there are differences in quality parameters across countries, while on the other hand, there is a concern more immediate within India that emerges from nonuniform interpretation of guidelines within the country. Further, there are complexities arising from the existing federal structure since states have differing regulatory capacities

which in turn are the result of insufficiency of trained personnel as well as testing capabilities. Toward addressing a number of such regulatory bottlenecks, dedicated efforts are required toward harmonizing the interpretation of legal terminology contained in the relevant act and guidelines, rationalizing the work distribution across regulatory personnel, using technological tools to bridge the gap in the data available to policymakers, and driving the industry toward voluntary compliance and self-regulation.

CONCLUSION

With the above discussion, the need for a quick and effective drug quality analysis method in developing countries like India stands quite clear. As shown from the experiments performed in this study, HPLC is not only a suitable but also efficient system to carry out drug quality evaluation and help curb or at least keep in check the rampant sale of substandard drugs that go unabated in developing countries like India.

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Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Morton LD, Pellock JM. Diagnosis and treatment of epilepsy in children and adolescents. Drugs 1996;51:399-414.
2. Heaney DC, Beran RG, Halpern MT. Economics in epilepsy treatment choices: Our certain fate? Epilepsia 2002;43 Suppl 4:32-8.
3. Krishnan RA, Chowdhury DJ. Cost of antiepileptic drugs in India. Neurology Asia 2007;12 Suppl 1:42-3.
4. Hanaya R, Arita K. The new antiepileptic drugs: Their neuropharmacology and clinical indications. Neurol Med Chir (Tokyo) 2016;56:205-20.
5. Levi L, Walker GC, Pugsley LI. Quality control of pharmaceuticals. Can Med Assoc J 1964;91:781-5.
6. Nikolin B, Imamović B, Medanhodzić-Vuk S, Sober M. High perfomance liquid chromatography in pharmaceutical analyses. Bosn J Basic Med Sci 2004;4:5-9.
7. Roy SMN, Yetal SM, Vaidya VV, Joshi SS. Determination and quantification of phenytoin in human plasma by liquid chromatography with electrospray ionization tandem mass spectrometry. J Chem 2008;5:169-76.
8. Das N, Dhanawat M, Shrivastava SK. An overview on antiepileptic drugs. Drug Discov Ther 2012;6:178-93.
9. Dharani S, Rahman Z, Barakh Ali SF, Afrooz H, Khan MA. Quantitative estimation of phenytoin sodium disproportionation in the formulations using vibration spectroscopies and multivariate methodologies. Int J Pharm 2018;539:65-74.
10. Teo SY, Rathbone MJ, Coombes AGA, Lee SY, Gan SN. Development and validation of a stability-indicating isocratic reverse phase-liquid chromatography assay for determination of phenytoin in bulk and pharmaceutical formulations. Int J Pharm Pharm Sci 2015;7:258-63.
11. Roin, B.N. (2014). Solving the problem of new uses by creating incentives for private industry to repurpose off-patent drugs. Harvard Law School. Available at: https://dash.harvard.edu/handle/1/11189865.
12. Johnston A, Holt DW. Substandard drugs: A potential crisis for public health. Br J Clin Pharmacol 2014;78:218-43.