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A Novel RP-HPLC Method Development and Validation for the Simultaneous Estimation of Sodium Benzoate and Sodium Phenylacetate in Pharmaceutical Formulations

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Research Article

A Novel RP-HPLC Method Development and Validation for the Simultaneous Estimation of Sodium Benzoate and Sodium Phenylacetate in Pharmaceutical Formulations

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Abstract

Sodium benzoate and Sodium phenylacetate were simultaneously estimated in pharmaceutical dosage forms by developing new, precise and accurate Reversed Phase – High Performance Liquid Chromatography (RP-HPLC) method. Chromatographic study was performed on C18 column using a mixture of methanol and potassium dihydrogen orthophosphate (0.05M) buffer in 60:40 v/v ratio with 1ml of triethylamine. The pH was adjusted to 5.5 using 1% ortho phosphoric acid. The peak response was monitored at 260nm after injecting the sample to HPLC system by marinating 1mL/min flow rate. The calibration curve was linear over the range of 5-25µg/mL for sodium benzoate and 5-25 µg/mL for sodium phenylacetate. The average Retention Time (RT) for sodium benzoate was found to be 3.366 ±0.0147 min and sodium phenylacetate was found to be 5.247±0.0354 min. Sodium benzoate recovery value was 99.9% and that of sodium phenylacetate was 100.16%. This confirms that the excipients of the formulation do not interfere during the analysis. Thus the projected RP-HPLC technique can be used for the estimation of these two drugs in pharmaceutical formulations simultaneously.

Key words: HPLC, Sodium benzoate, Sodium phenylacetate

Introduction

Sodium benzoate (SDBZ) (Fig 1) is a synthetic chemical produced when benzoic acid (which is found naturally in some fruits and spices) combines with sodium hydroxide. Since SDBZ contains natural ingredients, it is found safe[1]. SDBZ is a sodium salt that is present at extremely low levels in apples, plums, cinnamon and several other natural foods[2]. It is widely used as a preservative in several types of food including soft drinks, pickles, margarine, jelly and jams. Although SDBZ is safe, it may cause mild side effects in certain individuals like vomiting, stomach ulcer, mild hyperventilation, possibly leading to dizziness[3,4].

Sodium phenylacetate (SDPA) (Fig 2) is the sodium salt form of phenylacetate with ammonia detoxifying activity. Upon administration, SDPA binds to glutamine thereby forming phenylacetyl glutamine and is thus excreted by the kidneys[5], by aiding as a substitute to urea for the evacuation of waste nitrogen. SDPA is able to lower ammonia levels in the blood and prevent hyperammonemia[6].

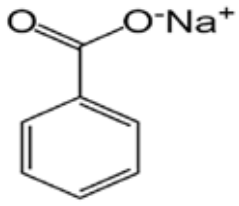

Figure 1: Structure of Sodium Benzoate

The combination of SDBZ and SDPA is available in aqueous solution form for injection (dissolved in water for injection). The pH of the solution

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lies between 6 and 8. It is indicated as adjunctive therapy in adult and pediatric patients for the treatment of acute hyperammonemia and associated encephalopathy in patients with deficiency in enzyme of the urea cycle[7-9].

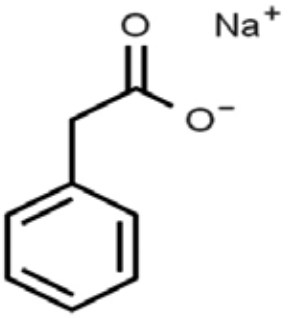

Figure 2: Structure of Sodium Phenylacetate

A few analytical techniques were spotted in literature survey for estimating these two drugs collectively and in combination with many other drugs[10-15]; however, there are no reports available for the estimation of the SDBZ and SDPA in pharmaceutical formulations simultaneously. Therefore, efforts are being made to establish fast, reliable and accurate RP-HPLC techniques for the evaluation of these drugs simultaneously, and to expand it in formulations for their determination.

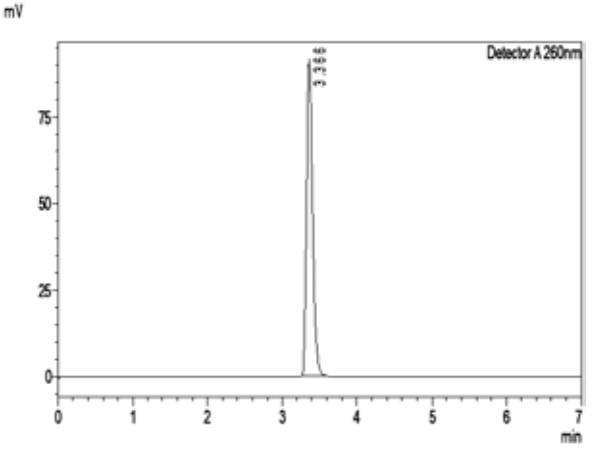

Figure 3: Chromatogram showing retention time of Sodium benzoate.

Materials and Methods

Chemicals and Reagents

The working standards of SDBZ and SDPA were given by Amneal Pharmaceuticals India Pvt Ltd (Ahmedabad, Gujarat, India). Ammonul, an injection, of Mylan Company claimed to contain 10 %/10% concentrated aqueous solution of sodium benzoate and sodium phenyl acetate, and was used in the analysis. Potassium dihydrogenortho phosphate, triethyl amine and o-phosphoric acid were obtained from Sd Fine chemicals Pvt Ltd, Mumbai. Methanol (HPLC grade) was obtained from Merck Ltd Mumbai, India.

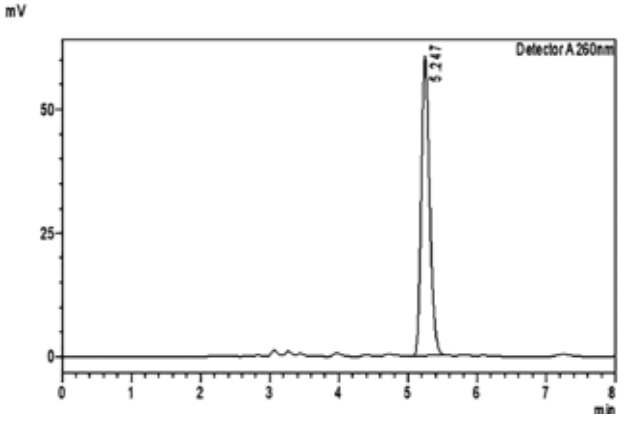

Figure 4: Chromatogram showing retention time of Sodium Phenyl acetate.

Instrumentation

A Shimadzu- HPLC (Japan) unit containing SPD-20AD UV-Visible detector; Enable C18 column (Shimadzu; Japan) having 250mmX4.6mmX5µm as specifications; LC-20 AD Pump; Quantitative HPLC had been done on an isocratic mode with 20 µL manual loop injection system. The results were combined using software Lab Solution.

Mobile phase preparation

The HPLC grade methanol was passed through 0.45µm filter and 0.05M of potassium dihydrogen orthophosphate in HPLC water (6.867g in 1000mL of water) was passed through 0.45µm membrane filter. The mobile phase was equipped by combining buffer, methanol and triethylamine in 600, 400 and 1 mL respectively mL and pH was adjusted to 5.5 with the help of ortho-phosphoric acid (OPA) and the blend was sonicated for 15 min.

Standard stock solution preparation

100 mg each of SDBZ and SDPA were measured individually and transferred into two separate 100mL volumetric flasks. Both the drugs were dissolved in

60mL of the mobile phase by sonication and then the volume was managed to make up to the mark with mobile phase to obtain a final concentration of 1000 µg/mL of each element (stock A and A' solution).

From the above stock A and A' of SDBZ and SDPA solution, 10 mL of aliquot was taken out in a standard flask (100 mL) and the volume was rendered up to the mark with mobile phase to acquire the final concentration of 100 µg/mL of SDBZ (stock B) and SDPA (stock B'). From the above stock B and B ' even more dilutions were performed to have a concentration range of 5-25 µg / mL for both the drugs.

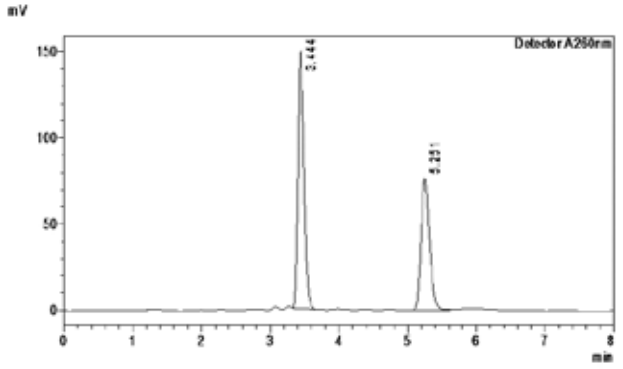

Figure 5: Chromatogram showing retention time of SDBZ and SDPA in formulation

Calibration curves

Appropriate additional dilutions were performed individually and 20 µL of each was pumped into the HPLC system and their chromatograms were obtained under the same chromatographic conditions as stated above. Peak areas for all peaks were documented and a standard Area Under Curve (AUC) calibration curve was graphed against concentration.

Chromatographic condition

The mobile phase having a mixture of buffer and methanol with the ratio of 60:40 v/v with 1mL of triethylamine at pH-5.5 was chosen as the best composition of the mobile phase, since it was found that this solvent system resolved both the components preferably. The flow rate was altered to 1 mL/min with the detection wavelength of 260 nm. Samples and mobile phase have been degassed for 15 min by sonication and screened through a membrane filter paper of 0.45µm. All assessments were held at steady column temp (25° C).

Selection of analytical concentration range

From the standard stock solution (solution B and B ') appropriate quantity was pipetted out into 10mL standard flasks. The quantity was rendered up to the 10mL mark in all the dilutions with the mobile phase to become a set of solutions giving the concentration range, varying from 5-25µg/mL for both SDBZ and SDPA respectively.

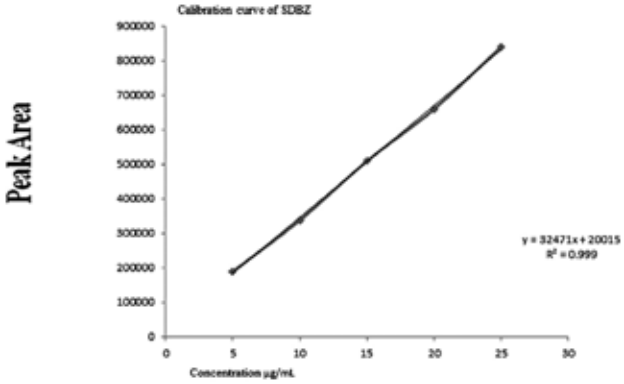

Figure 6: Calibration curve of SDBZ at 260 nm by RP-HPLC method

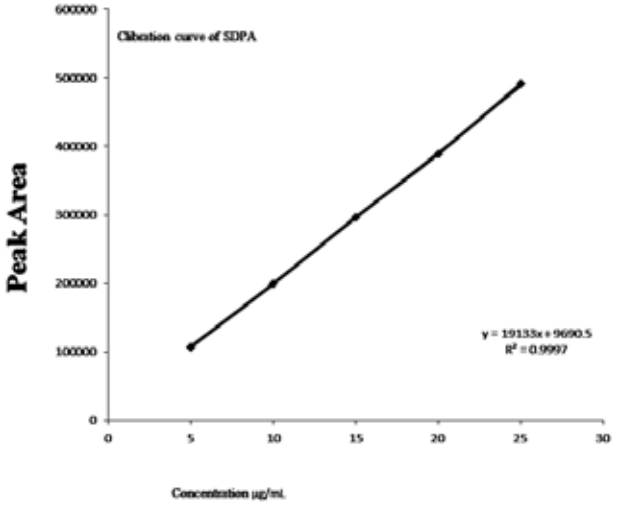

Figure 7: Calibration curve of SDPA at 260 nm by RP-HPLC method.

Triplicate dilutions for each of the above mentioned concentrations were made individually and from these solutions. From these solutions 20µL of the samples was pumped into the HPLC system twice distinctly and the chromatograms were documented under the similar chromatographic situations as mentioned in the above section (Fig

3 and 4 [1]). Peak areas were reported for all the experiments and the graph was plotted by taking concentration against standard calibration curve (Fig 6 and 7).

Table 1: Summary of validation and System suitability parameters of SDBZ and SDPA

Parameters	SDBZ	SDPA
Linear range (µg/mL)	5-25	5-25
Slope	32471	19133
Intercept	20015	9690.5
Regression coefficient (r^2)	0.999	0.9997
Limit of Detection (µg/mL)	0.0258	0.0226
Limit of Quantification (µg/mL)	0.0782	0.0685
Retention time (min)	3.366	5.247
Tailing factor	1.300	1.224
Theoretical plate (N)	6252	8137

Analysis of formulation

From the formulation, an amount having 100mg of SDBZ was verified experimentally and shifted to a 100mL volumetric flask, the quantity was made up to the mark by using the mobile phase to get 1000µg/mL (stock A). The materials were subjected to sonication for 15 min and the final volume was made up to the mark with the mobile phase. From the main stock (A) 10 mL was pipetted out and made up to 100 mL with mobile phase to get the 100µg/mL (stock B).

This solution was filtered through a 0.4 µm filter paper and was used as a standard stock solution. The suitable amount was pipetted out from the standard stock B and was further diluted with the same mobile phase to achieve a mixture having 10µg/mL of SDBZ and 10µg/mL of SDPA. A similar mixture having 10µg/mL of both SDBZ and SDPA was prepared as above from the standard stock solution. A 20µL volume of each sample mix was injected into the HPLC system and the chromatograms were documented (Fig 5). The AUC of each peak was calculated at 260nm and the amount of drug having in the sample mixture was calculated.

Method validation

Multiple validation variables such as the recovery studies, limit of detection (LOD), accuracy, linearity, the limit of quantification (LOQ), precision, and reproducibility were carried out for the established technique and their data were shown.

Table 2: Recovery of SDBZ and SDPA in spiked standard drug solution

Level of % recovery	Amount of formulation (µg)		Amount of standard drug added (µg)		Total amount recovered (µg)		% Recovery	
	SDBZ	SDPA	SDBZ	SDPA	SDBZ	SDPA	SDBZ	SDPA
80%	10	10	5	5	14.92	15.05	99.46	100.33
	10	10	5	5	15.1	14.92	100.66	99.46
	10	10	5	5	15.04	14.97	100.26	99.8
100%	10	10	10	10	20.05	20.08	100.25	100.4
	10	10	10	10	19.94	20.18	99.7	100.9
	10	10	10	10	19.89	20.16	99.45	100.8
120%	10	10	15	15	24.89	25.06	99.56	100.24
	10	10	15	15	24.92	24.94	99.68	99.76
	10	10	15	15	25.06	25.03	100.24	100.18

Table 3: Statistical Validation Data of Accuracy

Level of % Recovery	Mean of % recovery*		Standard Deviation*		Co-efficient of Variation*		Standard Error*	
	SDBZ	SDPA	SDBZ	SDPA	SDBZ	SDPA	SDBZ	SDPA
80%	100.13	99.86	0.6110	0.4371	0.6101	0.4377	0.3527	0.2524
100%	99.8	100.7	0.4092	0.2645	0.4100	0.2627	0.2362	0.1527
120%	99.82	99.96	0.3629	0.2498	0.3635	0.2498	0.2095	0.4421

*n=3

Table 4: Intra day Precision of SDBZ and SDPA

Sr no	Amount present (µg/mL)		Amount obtained(µg/mL)		% Obtained	
	SDBZ	SDPA	SDBZ	SDPA	SDBZ	SDPA
1	15	15	14.87	15.03	99.13	100.2
2	15	15	15.04	15.1	100.26	100.66
3	15	15	14.94	15.04	99.6	100.26
4	15	15	14.98	14.97	99.86	99.8
5	15	15	14.92	15.1	99.46	100.66
6	15	15	15.07	14.95	100.46	99.66

Table 5: Statistical Validation Data for Intra day Precision

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
SDBZ	99.8	0.5024	0.5034	0.2051
SDPA	100.21	0.4204	0.4195	0.1716

*n=6

Table 6: Inter day Precision of SDBZ and SDPA

Sr No	Amount present (µg/mL)		Amount obtained (µg/mL)		% Obtained	
	SDBZ	SDPA	SDBZ	SDPA	SDBZ	SDPA
DAY – 1						
1	15	15	14.97	15.03	99.8	100.2
2	15	15	15.04	14.99	100.26	99.93
3	15	15	15.05	15.03	100.33	100.2
4	15	15	15.07	15.08	100.46	100.53
5	15	15	14.89	14.94	99.26	99.6
6	15	15	14.9	15.05	99.33	100.33
DAY – 2						
1	15	15	15.02	15.03	100.13	100.13
2	15	15	14.97	14.96	99.8	99.7
3	15	15	14.99	14.98	99.93	99.86
4	15	15	14.93	15.03	99.53	100.2
5	15	15	15.07	15.08	100.46	100.53
6	15	15	15.04	14.99	100.26	99.93
DAY – 3						
1	15	15	14.99	15.05	99.93	100.33
2	15	15	14.89	15.07	99.26	100.46
3	15	15	14.94	15.02	99.6	100.13
4	15	15	14.98	14.99	99.86	99.6
5	15	15	15.03	14.96	100.2	99.73
6	15	15	15.05	14.89	100.33	99.26

Table 7: Statistical Validation Data for Inter day Precision

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
SDBZ	99.86	0.3910	0.3915	0.1596
SDPA	99.92	0.4645	0.4649	0.1896

Table 8: Robustness results for variations in detection wavelength

Method Parameter	Level	Retention Time		Tailing factor	
Wavelength (nm)		SDBZ	SDPA	SDBZ	SDPA
258	+2	3.359	5.238	1.311	1.227
260	0	3.366	5.242	1.300	1.224
262	-2	3.372	5.249	1.303	1.229

Table 9: Robustness results for variations in flow rate (mL/min)

Method Parameter	Level	Retention Time		Tailing factor	
Flow Rate (mL/min)		SDBZ	SDPA	SDBZ	SDPA
0.8	-0.2	3.375	5.250	1.310	1.220
1	0	3.366	5.242	1.300	1.224
1.2	+0.2	3.355	5.234	1.306	1.227

Results and Discussion

The present analysis negotiates with the simultaneous measuring of SDBZ and SDPA in mixed pharmaceutical dosage form by RP-HPLC technique. The method which is established is based on the estimation of both the test substances by determining the area at the desired analytical wavelength under the chromatogram curve. An analysis of calibration curve on at least square regression determined the linearity of the proposed operation. The constructed calibration curves have been linear over the concentration range of 5-25µg/mL. The r2 values are with 0.999 and 0.9997 respectively for SDBZ and SDPA, along with the overview of validation and system suitability parameters as mentioned in the Table 1. Recovery experiments were also conducted to assess the accuracy and precision of the current method. Recovery studies were conducted at three different levels, 80%, 100% and 120% of the labeled amount of both the drugs as shown in Table 2. 3 replicate samples of each concentration level were operated, and the percentage recovery at each level (n = 3) and mean percentage recovery (n = 3) were measured, and the accuracy determination quantitative validation data outlined in Table 3 were calculated.

Intra-day precision as calculated by assaying samples comprising 15µg/mL of SDBZ and 15µg/mL of SDPA for six times and the outcomes were scored for statistical analysis. The statistical validation data and assay results for intra-day precision are outlined in Table 4 and 5. Inter-day precision was measured by evaluating a set of quality control samples comprising 15µg/mL of SDBZ and 15µg/mL of SDPA, replicates were examined on three days in a row. The determination of inter-day precision and statistical validation data for inter-day precision is outlined in Table 6 and 7. All intra-day and inter-day deviation showed less percentage Relative Standard Deviation (%RSD) value specifying a high grade of precision of the technique. The robustness was assessed by analyzing the samples by differing few variables like wavelength and flow rate. The commitment of robustness is summarized in Table 8 and 9.

From the outcomes of validation, it is clear that the developed RP-HPLC method is simple, accurate and precise for the analysis of SDBZ and SDPA in pharmaceutical dosage forms. This technique does not need much prior segregation of SDBZ and SDPA before analysis. In addition, it is appropriate for implementation without the interference of excipients and can be easily transferred to the commercial batches without previous treatment.

Conclusion

The current study defines the development and validation of a new RP-HPLC technique for the calculation of SDBZ and SDPA in combination using simple mobile phase. The process was discovered to be very easy, accurate, sensitive, and precise. So the established technique can be used easily

for evaluation of SDBZ and SDPA in combined pharmaceutical products.

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References

1. Sigma-Aldrich Co., Sodium benzoate. Retrieved on 2014. www.sigmaaldrich.com.
2. Cosmetic Ingredient Review Expert Panel Bindu Nair. "Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate". Int. J. Tox., 2001; 20 (3): 23–50.
3. Lewis RJ. Hawley's Condensed Chemical Dictionary 14th Edition. John Wiley & Sons, Inc. New York, NY 2001; 1021.
4. Novak KM. Drug facts and comparisons. Wolters Kluwer Health. St. Louis, Missouri 2005;59th Ed.,535.
5. Brusilow SW, Danney M, Waber LJ, Batshaw M, Burton B, Levitsky L, Roth K, McKeethren C, Ward J. Treatment of episodic hyperammonemia in children with inborn rrors of urea synthesis. New Engl. J. Med., 1984; 310(25):1630-1634.
6. Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor AO, Tompkins AC, Weinberger MS, Headlee DJ, McCall NA, Samid D, Myers CE. A Phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res., 1994;54:1690-1694.
7. Summar M, Tuchman M. Proceedings of a consensus conference for the management of patients with urea cycle disorders. J. Pediatr., 2001; 138 (1): S6-10.
8. Summar M. Current strategies for the management of neonatal urea cycle disorders. J. Pediatr., 2001; 138(1): S30-9.
9. The urea cycle disorders conference group. Consensus statement from a conference for the management of patients with urea cycle disorders. J. Pediatr., 2001; 138 (1): S1-5.
10. Balachandran S, Sudha DR. Estimation of sodium benzoate in ayurvedic formulation: Kashaya (Water Decoction). Asi. J. Chem., 2007; 19 (5); 3421-26.
11. Grether MT. Rapid high-performance liquid chromatography for the analysis of sodium benzoate and potassium sorbate in foods. J. Chromatogr., A, 2000; 883 (1-2): 299–304.
12. Venkata SK, Ravishankar K, Peer BD, Kiranmayi GVN. Estimation of caffeine and sodium benzoate in caffeine and sodium benzoate injection by isoabsorption method (isobestic method). IJPCBS, 2011; 1(1): 26-31.
13. Robert BMA, Arman A, Mendel T. Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers. Mol. Gen. Metabol., 2004; 81: 67-73.
14. Liana A, Michel C, Claire S, Lucien I. Sodium phenylacetate induces growth inhibition and Bcl-2 down-regulation and apoptosis in MCF7ras cells in vitro and in nude mice. Ame. Asso. can. Res., 2018.
15. Inderjit S ,Kalipada P, MushfiquddinK. Lovastatin and sodium phenylacetate normalize the levels of very long chain fatty acids in skin fibroblasts of X adrenoleukodystrophy. FEBS Lettr.,1998;426 (3):342-46.