Drug Testing and Analysis

Published online in Wiley Interscience:

(www.drugtestinganalysis.com) DOI 10.1002/dta.28

Preservatives in liquid pharmaceutical preparations†

Introduction

Preservatives have been used commonly as additives in pharmaceutical products, cosmetic, and food. Liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredients. Such preparations are protected by the addition of preservatives, which prevent the alteration and degradation of the product formulation.[1] Preservatives are mainly effective in controlling mould, inhibiting yeast growth and preventing bacterial proliferation. Their antimicrobial and antifungal properties make them an integral part of the product formulation. Amongst the most commonly used preservatives in the conservation of liquid pharmaceutical preparations are sodium benzoate, potassium sorbate and methyl hydroxybenzoate (methylparaben). The typical concentrations of these substances allowed are from 0.1% to 0.2%, from 0.1 to 0.2% and from 0.1% to 0.25% (w/w) respectively.[1] The required amount with the pH, dissociation and the presence of other formulative ingredients with inherent preservative capabilities. The purpose of this study is to determine the amount of the former preservatives in liquid pharmaceuticals for quality assurance purposes as well as for patient safety.

Interest is given to preservatives because recent studies have reported serious side effects associated with these substances. Skin reactions such as rash, urticaria, and contact dermatitis have been reported after topical application of products containing potassium sorbate, methylparaben and sodium benzoate.[1,2,3] Other side effects have been reported after ingestion of medications containing these preservatives, such as allergic and estrogenic effects of parabens[2,4] or genotoxic effects of sodium benzoate.[3]

Experimental

Thirty-seven liquid pharmaceutical preparations were purchased from a randomly selected pharmacy in Lebanon. Not all preparations had labels showing their preservative content. Nineteen preparations had labels indicating their methylparaben content, eight had labels showing their sodium benzoate content and one had a label indicating its potassium sorbate content. Fourteen preparations were unlabelled with respect to their preservative content. Commercial brands of the liquid preparations were coded using letters (A to AL).

Acetonitrile (HPLC grade) and sodium acetate (analytical grade) were purchased from Sigma, Germany. Sodium benzoate, potassium sorbate and methylparaben standards were purchased from Fluka, UK. Standards were used to prepare standard working solutions using distilled water, to obtain stock solutions of 1 g/L concentration. Stock solutions were used to prepare solutions of lower concentrations (50, 100, 200 and 400 mg/L) to build the calibration curve. The correlation coefficients were above 0.999 in all cases.

Preparations were analysed in triplicate for their preservative content using a sensitive and reproducible HPLC method[5–8] modified in our laboratory. The HPLC system consisted of LC-10 pump (Shimadzu), a variable ultraviolet detector monitor set at 229 nm (Shimadzu, SPD-10) and a Chromatopac Shimadzu (C-R8A) integrator. A pre-packed stainless-steel column (15 cm $\times$ 0.46 cm) filled with Shimpack C18 10 µm Silica (Waters, Germany) was used for separation and the flow rate was set to 1.5 mL per minute for sodium benzoate and potassium sorbate and 2 mL/min for methylparaben. The precision of the assay method was determined by calculating the relative standard deviation (inter- and intra-days) of the peak areas obtained after repeated injections ($n = 3$) of all standard solutions. The relative standard deviations of the areas were found to be less than 3.5%, which confirms the precision of the method.

One gram of each of the samples was accurately weighed and transferred to a 100 mL volumetric flask. Mobile phase was added to volume and mixed. The mobile phase was an aqueous solution of acetonitrile 0.02 M sodium acetate buffer (20 : 80, v/v), adjusted to a pH 4.3 with acetic acid. The mobile phase was filtered through a 0.45 µm filter and degassed before use. Twenty µL of the filtrate was injected into the HPLC.

The retention times for sodium benzoate, potassium sorbate and methylparaben were 5.2, 6.7 and 7.6 minutes, respectively.

Results and Discussion

In the eight samples labelled as containing sodium benzoate as a preservative, concentrations of the latter ranged between 0.012% and 0.85% (w/w). Two samples (A and G) included sodium benzoate in amounts significantly exceeding the typical allowed concentrations (up to a fourfold difference) whereas two other products had a sodium benzoate concentration slightly exceeding that range (Figure 1).

After screening the 14 unlabelled samples, six samples were found to contain sodium benzoate in concentrations ranging from 0.05% to 0.48% (w/w). Only Product K contained the preservative in concentrations above the typical allowed concentrations (Figure 2). Three products exhibited sodium benzoate concentrations below typical allowed concentrations. As much as 50% of the pharmaceutical products containing sodium benzoate are formulated at a pH > 5 at which the preservative is ineffective.

As for potassium sorbate, the only labelled product included a higher amount of preservative. Many unlabelled samples were found to contain potassium sorbate as a preservative. As shown

* *Correspondence to:* , Soula Kyriacos. E-mail: soula.boustani@lau.edu.lb

† *Articles in this section are subject to screening for scientific accuracy but do not undergo the traditional model of peer review.*

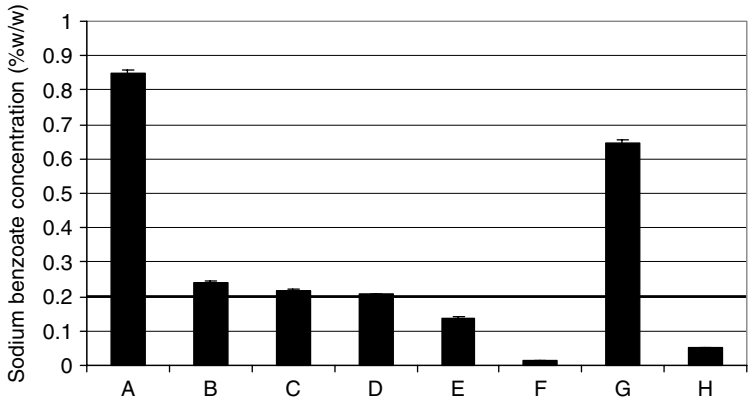

Figure 1. Concentration (%w/w) of sodium benzoate in labelled samples. (Upper limit of the allowed concentration range is shown).

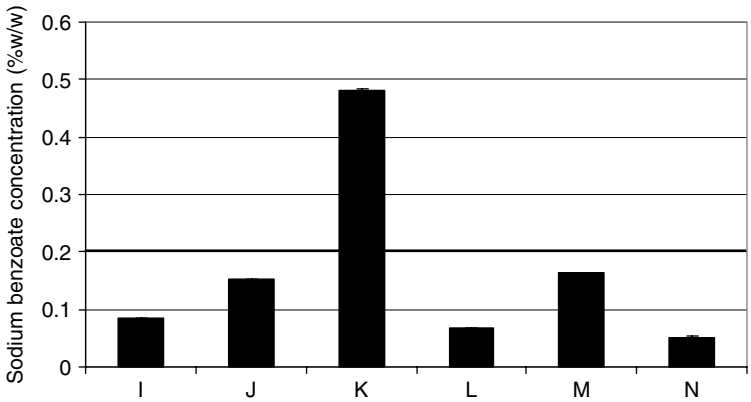

Figure 2. Concentration (%w/w) of sodium benzoate in unlabelled samples.

in Figure 3, products E and O exceeded the typical concentrations allowed for its use.

Nineteen samples were labelled as having methylparaben as a preservative. It was found in concentrations ranging from 0.03% to 0.55% (w/w). Only one labelled product included methylparaben in a concentration exceeding the maximum allowed concentration and 14 out of the 19 samples had concentrations below the allowed range (Figure 4).

None of the unlabelled samples exhibited concentrations exceeding the typical allowed range (Figure 5). All, however, had methylparaben concentrations below the allowed range.

Conclusion

Preservative levels were found to fall outside the typical allowed concentration range for 70% of the samples, with some exhibiting significant higher concentrations. The present study highlights the large amounts of preservatives that may be found in some liquid pharmaceutical preparations. The reason behind this finding is unclear; it could be due to poor quality control or to intentional attempts to extend the shelf-life of the products. Consequences for patients' health need to be evaluated, especially because most liquid pharmaceutical products are administered to the paediatric

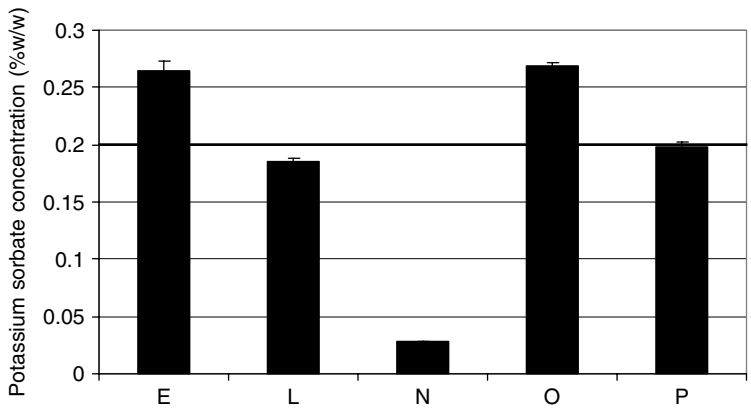

Figure 3. Concentration (%w/w) of potassium sorbate in labelled (sample E) and unlabeled samples (samples L,N,O,P).

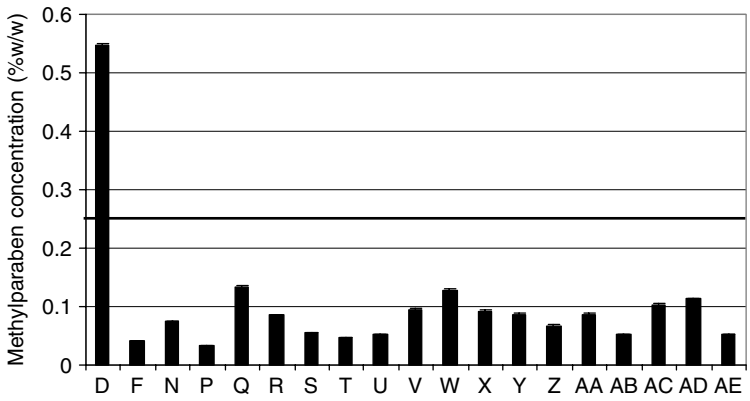

Figure 4. Concentration (%w/w) of methylparaben in labelled samples.

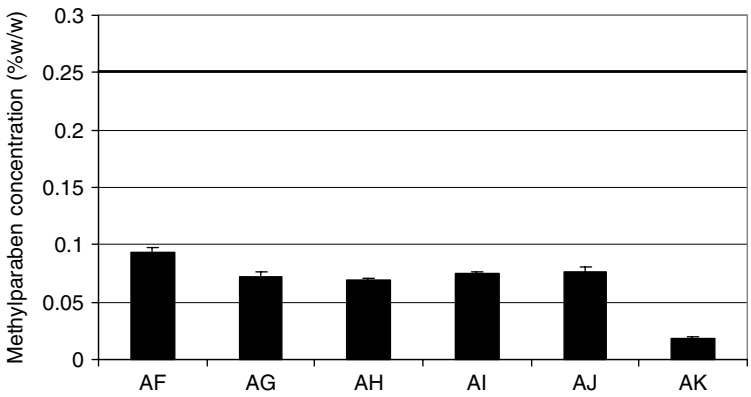

Figure 5. Concentration (%w/w) of methylparaben in unlabeled samples.

population. Labelling of preservatives should be mandatory and concentrations present in pharmaceutical products should be rigorously controlled as they may expose some patients to adverse reactions.

Acknowledgements

This work was supported by a grant from the Research Council at the Lebanese American University.

Yours,

CHAWKI BOUKARIM, SARAH ABOU JAOUDE, RITA BAHNAM, ROULA BARADA, SOULA KYRIACOS*
Lebanese American University, Pharmaceutical Sciences

References

[1] P. Beringer, A. derMarderosian, L. Felton, S. Gelone and A. R. Gennaro *Remington: The Science and Practice of Pharmacy*, 21st edition, Lippincott Williams & Wilkins: Philadelphia, **2006**.
[2] M. G. Soni, I. G. Carabin, G. A. Burdock, *Food Chem. Toxicol.* **2005**, *43*(7), 985.
[3] B. Nair, *Int. J. Toxicol.* **2001**, *20*(Suppl 3),, 23.
[4] M. G. Soni, S. L. Taylor, N. A. Greenberg, G. A. Burdock, *Food Chem. Toxicol.* **2002**, *40*(10), 1335.
[5] G. Shabir, *J. Pharm. Biomed. Anal.* **2004**, *34*, 207.
[6] D. Satinsky, J. Huclova, R. L. C. Ferreira, *et al*., *J. Pharm. Biomed. Anal.* **2006**, *40*, 287.
[7] H. M. Pylypiw Jr., M. T. Grether, *J. Chromatogr. A.* **2000**, *883*, 299.
[8] K. Kuprovskyte, B. Pranaityte, A. Padarauskas, *Chemija (Vilnius)*. **2002**, *T. 13*, 3.