

## Mini-Review on Various Analytical Methods for Determination of Certain Preservatives in Different Matrices

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### ABSTRACT

In this literature mini-review, we will introduce mode of action and most of up-to-date reported methods that have been developed for determination of certain preservatives such as methylparaben, propylparaben, benzoic acid and potassium sorbate in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples. Most of reported methods include spectrophotometric and chromatographic methods in addition to some electrochemistry methods.

**Keywords:** mini-review; preservatives; methylparaben; propylparaben; benzoic acid; potassium sorbate.

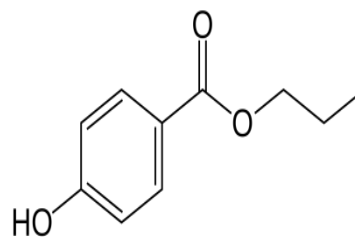
### INTRODUCTION

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, Propyl Hydroxybenzoate (Propylparaben) 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].

As such, in this mini-review article, four preservatives have been studied in respect of physical, chemical characters, mode of action and most reported analytical methods that have

been developed for determination of these drugs in different matrices.

### Methylparaben (Mpn)



### Chemical Name

Methyl 4-hydroxybenzoate [2].

### Molecular Formula

C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>

### Molecular Weight

152.1 gm/mol.

### Physical Properties

White or almost white, crystalline powder or colorless crystals. It is very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol [2].

**Melting point**

131°C [3].

**METHODS OF DETERMINATION**

**Official Methods**

The BP proposes a HPLC-UV method using 0.15 m end-capped octadecyl silyl silica gel for chromatography column and mobile phase of 35:65 V/V 6.8 g/L solution of potassium dihydrogen phosphate, methanol, the detection was at 272 nm [2].

The USP proposes a non-aqueous titration for the determination of MPN. The drug was dissolved in 1N sodium hydroxide followed by addition of methyl ethyl ketone then titration with 1 N sulfuric acid, determining the end-point potentiometrically [4].

**Spectroscopic Methods**

Literature describes different spectroscopic methods for determination of MPN. A simple method was developed for the determination of MPN via oxidative coupling with the 2,4-Dinitrophenyl hydrazine in the presence of N-bromosuccinimide in a basic medium, Absorbance of the obtained colored products was measured at the corresponding optimum wavelengths (600 nm) [5].

**Chromatographic Methods**

Several chromatographic methods were described for the determination of the proposed drug either in pure or in combination with other drugs summarized in Table 1.

**Table 1.** Chromatographic methods for the determination of MPN in pure form or in combination with other drugs.

Drugs	Method	Column	Mobile phase	Detector	λ	Ref
MPN, Ethylparaben, Nipa-sol and Benzalkonium	HPLC	Hypersil GOLD C <sub>18</sub> column	0.005 mol/L ammonium acetate (10 mL triethylamine in 1 L solution, pH adjusted to 5.0±0.5 with glacial acetic acid)-acetonitrile (45:55, V/V)	UV	262 nm	[6]
MPN, Ethylparaben, Propylparaben, and Butylparaben	RP-HPLC	Phenomenex Gemini C <sub>18</sub> (5 cm×4.6 mm, 5 μm)	Acetonitrile: water (34:66, v/v),	UV	254 nm	[7]
Methylisothiazolinone, Methylchlorisothiazolinone, Benzyl alcohol, Sodium benzoate and MPN	HPLC	(50 × 2 mm i.d.; Merck)	Included water, methanol, acetonitrile, a 0.05% aqueous solution of trifluoroacetic acid, a 0.1% aqueous solution of formic acid and mixtures thereof.gradient elution	UV	257 nm	[8]
2-phenoxyethanol, MPN, Ethylparaben and Propylparaben	RP-HPLC	Lichrosorb C <sub>8</sub> (150×4.6 mm, 5 μm) column	Mixture of acetonitrile, tetrahydrofuran and water (21:13:66, v/v/v),	UV	258 nm	[9]
MPN, Ethylparaben, Propylparaben and Butylparaben	GC-MS	An Rtx-5MS capillary column	Helium	Mass spectroscopy	MPN: 151, 121 Propylparaben: 180, 138 m/z	[10]
Ketoprofen, MPN, and Propyl Paraben	HPTLC	Silica gel 60 F <sub>254</sub> Merck plates	Toluene:Ethyl acetate:Glacial acetic acid (6.5:2.5:1.0 v/v/v)	UV	265 nm	[11]
MPN - propylparaben, Propylparaben -	HPTLC	Pre-coated HPTLC plates,	n-pentane - glacial acetic acid (88:12, v/v)	densitometry	256 nm	[12]

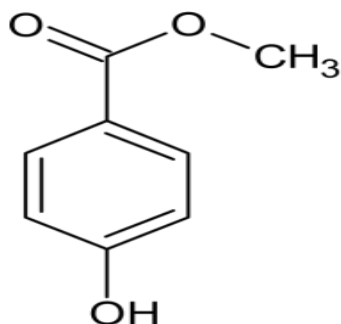
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Butylparaben		silica gel 60F <sub>254</sub> for nano-TLC, 10x10 cm, Merck, West Germany.				
Potassium sorbate, MPN, Propylparaben	HPLC	Eclipse XDB-C8 column	Acetonitrile:citrate buffer, pH 4.0 (20:80 v/v)	UV	260 nm	[13]
Betamethasone-17-valerate, Fusidic acid and Potassium sorbate as well as Methyl- and Propylparaben	RP-HPLC	A 100 mm × 3.0 mm ID. Ascentis Express C <sub>18</sub> column	Mobile phase A comprised of an 83:17 (v/v) mixture of acetonitrile and methanol and mobile phase B of a 10 g/l solution of 85% phosphoric acid in purified water	UV	240 nm	[14]
Ethylparaben, MPN and Propylparaben	RP-HPLC	Spherisorb C <sub>18</sub> column (250mm × 4.6 mm)	Potassium phosphate buffer (pH7.05)-methanol (47.5:52.5, v/v)	UV	254 nm	[15]
Levetiracetam, MPN and propylparaben	RP-HPLC	Equisil BDS, C <sub>18</sub> , 5 μm, (150 mm × 4.6 mm)	NaH <sub>2</sub> PO <sub>4</sub> : Methanol in ratio of (55:45) pH 7.70 by NaOH	UV	254 nm	[16]

### Miscellaneous Methods

A voltammetric method using gold electrode was used for the electroanalytical determination of MPN in a pharmaceutical product and urine sample by cyclic, linear sweep and square-wave voltammetric techniques. The oxidation of MPN is irreversible and exhibits a diffusion-controlled process. The oxidation mechanism was proposed. The dependence of the current on pH. It was found that the optimum buffer for the determination of MPN is pH of 7.0 [17]. A method was developed for the determination of MPN using modified glassy carbon electrode based on a synthesized polypyrrole by differential pulse voltammetry. polypyrrole was covalently immobilized on glassy carbon electrode surface using tetraethyl ammonium perchlorate and acetonitrile. MPN was oxidized by differential pulse voltammetry and a peak was observed at 1.20 V [18].

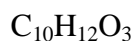
### PROPYLPARABEN (PPN)



### Chemical name:

Propyl 4-hydroxybenzoate. [2]

### Molecular formula



### Molecular weight

180.2 gm/mol.

### Physical properties

White or almost white, crystalline powder. It is very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol [2].

### Melting point

96-99°C [19].

### METHODS OF DETERMINATION:

#### Official methods

The BP proposes a HPLC-UV method using 0.15 m end-capped octadecyl silyl silica gel for chromatography column and mobile phase of 35:65 V/V 6.8 g/L solution of potassium dihydrogen phosphate, methanol, the detection was at 272 nm [2].

The USP proposes a non-aqueous titration for the determination of PPN. The drug was dissolved in 1N sodium hydroxide followed by addition of methyl ethyl ketone then titration with 1 N sulfuric acid, determining the end-point potentiometrically [4].

### Chromatographic Methods

Several chromatographic methods were described for the determination of the proposed

drug either in pure or in combination with other drugs summarized in Table 2.

**Table 2.** Chromatographic methods for the determination of PPN in pure form or in combination with other drugs.

Drugs	Method	Column	Mobile phase	Detector	$\lambda$	Ref.
MPN, Ethyl, Propyl and Butyl parabens	HPLC-DAD	Agilent Zorbax SB C <sub>18</sub> narrow bore RR	Acetonitrile:water (50:50: v/v)	UV	254 nm	[20]
MPN and Propyl paraben	HPLC	Waters 2489), column	Buffer (pH 2.0) and acetonitrile (68: 32)	UV	205 nm	[21]

### Miscellaneous Methods

A selective and sensitive method was successfully developed for PPN determination in cosmetic sample using voltammetric sensor for PPN based on a nanosized molecularly imprinted polymer-carbon paste electrode [22].

water, soluble in boiling water, freely soluble in ethanol (96 per cent) and in fatty oils [2].

### Melting Point:

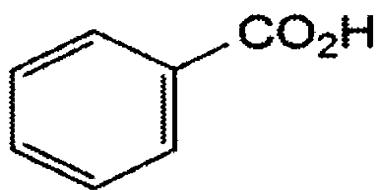
121-125 °C [2].

### METHODS OF DETERMINATION

#### Official Methods

The BP [2] and the USP [4] proposes an acid base titration for the determination of BZA. The drug was dissolved in 20 mL of ethanol (96 per cent) then titrated with 0.1 M sodium hydroxide, using 0.1 mL of phenol red solution as indicator, until the color changes from yellow to violet-red.

### BENZOIC ACID (BZA)



#### Chemical Name

Benzene carboxylic acid [2].

#### Molecular Formula

C<sub>7</sub>H<sub>6</sub>O<sub>2</sub>

#### Molecular Weight

122.1 gm/mol.

#### Physical properties

White or almost white, crystalline powder or colorless crystals. It is slightly soluble in

#### Spectroscopic Methods

Literature describes different spectroscopic methods for determination of BZA in [23-25].

#### Chromatographic Methods

Several chromatographic methods were described for the determination of the proposed drug either in pure or in combination with other drugs summarized in Table 3.

**Table 3.** Chromatographic methods for the determination of BZA in pure form or in combination with other drugs.

Drugs	Method	Column	Mobile phase	Detector	$\lambda$	Ref.
Salicylic, 3-methyl Salicylic, 4-methyl Salicylic, Acetylsalicylic and BZA	HPLC/	Phenomenex (Torrance, CA, USA) Kinetex C <sub>18</sub> , 4.6 mm x 100 mm (2.6 $\mu$ m).	Acetonitrile-phosphate buffer (pH 2.8; 2 mM) mixture (70:30, v/v)	DAD	220–380 nm range	[26]
Sorbic and BZA	HPLC-UV	PerfectSil Target C <sub>18</sub> that was purchased	40% Acetonitrile and 60% ammonium acetate buffer (5	UV	254 nm	[27]

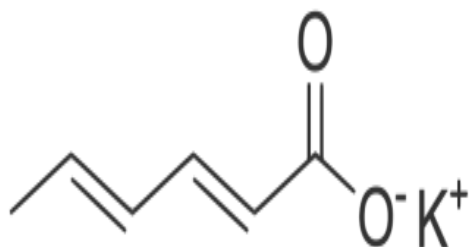
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		from Merck (250 × 4.6 mm, 5 μm)	mmole/L;pH 4.4)			
BZA, Sorbic acid, MPN and PPN	RP-HPLC	Jasco PV-1580 HPLC unit using a Supelco 516 C <sub>18</sub> column (15 cm×4.6 mm, 5_μm)	Methanol–acetate buffer (pH 4.4) (35:65) methanol–acetate buffer (pH 4.4) (50:50) thereafter.	UV	254 nm	[28]
Sodium benzoate and Potassium sorbate	HPLC	Supelcosil LC-C <sub>18</sub> , 25 cm×4.6 mm, 5 mm, Supelco, Bellefonte, PA, USA.	90% ammonium acetate buffer with 10% acetonitrile	UV	225 nm & 255 nm	[29]
BZA and Hippuric acid	HPLC	Yanapak ODS-A column (250 mm x 4,6 mm I.D., 7-μm particles, Yanagimoto, Kyoto, Japan).	Acetonitrile-water-acetic acid (35:63:2, v/v/v)	UV	235 nm.	[30]
Risperidone and BZA	RP-HPLC	Equisil BDS, C <sub>18</sub> , 5 μm, (150 mm × 4.6 mm)	0.05 M potassium dihydrogen phosphate (pH 2.5) and acetonitrile (65:35; v/v)	UV	260 nm	[31]

### Miscellaneous Methods

An electro kinetic flow analysis–ion pair solid phase extraction–capillary zone electrophoresis method for the determination of BZA and sorbic acid in food products has been reported [32].

### Potassium Sorbate (SRT)



### Chemical Name

Potassium 2,4-hexadienoate [2].

### Molecular Formula

C<sub>6</sub>H<sub>7</sub>KO<sub>2</sub>

### Molecular Weight

150.2 gm/mol.

### Physical Properties

White or almost white, powder or granules. It very soluble in water, slightly soluble in ethanol (96 per cent) [2].

### Melting Point

270 °C [33].

### METHODS OF DETERMINATION:

#### Official Methods

The USP and BP proposes a non-aqueous titration for the determination of SRT.

The drug was dissolved in glacial acetic acid, and titrated with 0.1 N perchloric acid VS to a blue-green endpoint using add 1 drop of crystal violet as indicator [2][4].

#### Chromatographic Methods

Several chromatographic methods were described for the determination of the proposed drug either in pure or in combination with other drugs summarized in Table 4.



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**Table 4.** Chromatographic methods for the determination of SRT in pure form or in combination with other drugs.

Drugs	Method	Column	Mobile phase	Detector	$\lambda$	Ref.
Sodium benzoate and SRT	HPLC–UV	ACE-121-1504 C <sub>18</sub> HPLC column (15 cm × 3.9 mm I.D., 5 $\mu$ m)	Ammonium acetate buffer:acetonitrile (72:28,v/v).	UV	225 and 255 nm	[34]
	LC–MS/MS	Phenomenex 150 × 2 mm, 4 l HPLC column.	Ammonium acetate buffer:methanol (50:50, v/v).	Mass spectroscopy		
SRT and Sodium benzoate	RP-HPLC	C <sub>18</sub> - bonded monolithic silica column	Phosphate buffer (0.025 M, pH 2.0)–water–acetonitrile (50:45:5, v/v/v)	UV	230 nm	[35]

### Miscellaneous methods

A new potentiometric sensor, namely, Pt|Hg|Hg<sub>2</sub>(SOB)<sub>2</sub> Graphite, where SOB stands for sorbate ion, are described constructed, evaluated and applied. The potentiometric response is independent of the pH of the solution in the pH range 6.0–9.0. The electrode was easily constructed at a relatively low-cost with fast response time (within 15–30 s) and can be used for a period of 4 months without significant change in its performance characteristics. The proposed sensor displayed good selectivities over a variety of other anions (carboxylates and inorganic anions).

The potentiometric sensor was successfully applied to the determination of sorbic acid in real food samples, that is, soft drinks, skim yogurts, jams and sauces [36].

### CONCLUSION

This literature mini-review represents an up to date survey about all reported methods that have been developed for determination of certain certain preservatives such as methylparaben, propylparaben, benzoic acid and potassium sorbate in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, and electrochemistry.

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