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Analytical Method of Pyridine-triphenylborane by High Performance Liquid Chromatography

- Study of reversed-phase column and ion-pair reagent -

Kazuhiko KITAORI, Misaki AKIYAMA
Central Research Laboratories, Hokko Chemical Industry Co., Ltd.
2165, Toda, Atsugi-shi, Kanagawa 243-0023 Japan

Eiichi YOSHIKAWA, Mizuho HONMA
Chugoku Marine Paints Co., Ltd.
1-7, Meijishinkai, Ohtake-shi, Hiroshima 739-0652 Japan

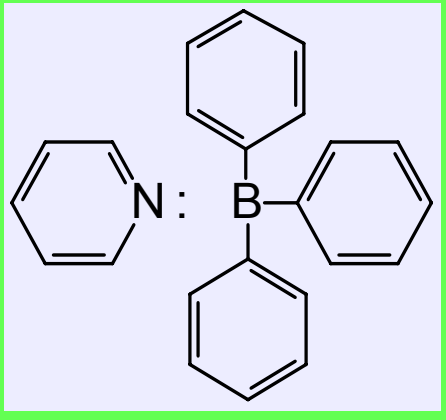
Kazunobu TAKAHASHI
Marine Antifouling & Environment Consultant
2-6-13-1008, Shigita-higashi, Jyoto-ku, Osaka 536-0017 Japan

Introduction

Pyridine-triphenylborane (PTPB) is used widely to commercial copper-free self-polishing antifouling paints.
ISO/TC35/SC9/WG27 (ISO/WG27) is going forward to establish the standard method for measuring the release rate of biocides from antifouling paint 1).
ISO/WG27 Japan working group associated with Japan Paint Inspection and Testing Association (JPIA) is going forward to develop the standard method for measuring the release rate of PTPB 2-3).
The analytical method of PTPB was drafted with a high performance liquid chromatography (HPLC) using a reversed-phase column and mobile phase containing 0.5 mol/l tetra-n-butyl ammonium phosphate (TBA-P) as ion-pair reagent. It was reported that proper peak of PTPB was not obtained by the draft using TBA-P supplied form Acros Organics and Nova-Pak® C18 (Waters) as HPLC column.
To clarify the amphibiotic test result, we have investigated the different five resources of TBA-P and eight kinds of the HPLC column in three laboratories.

Test substance

Pyridine Triphenylborane (PTPB)



CAS No.	971-66-4
Molecular formula	$C_{23}H_{20}BN$
Molecular weight	321.2
Appearance	White to slightly yellowish powder
Specific gravity	1.14
Melting point	210ºC (decompose)
Solubility (at 20ºC)	Water 0.08 mg/L

PTPB standard: purity 99.1%,
Hokko Chemical Industry Co., Ltd. (Tokyo, Japan).

Ion-pair reagent

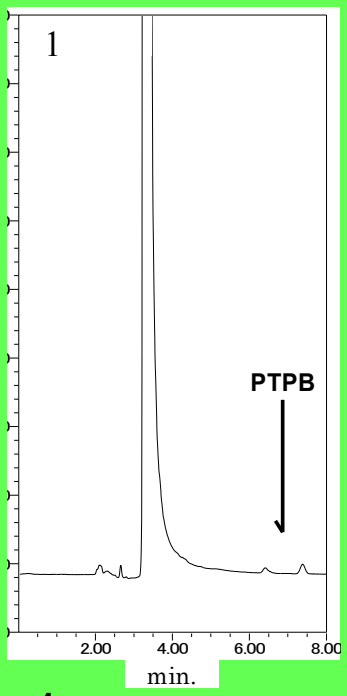
Table 1) The shape of PTPB peak and ion-pair reagent

Product	pH of mobile phase	Shape of PTPB peak	Sensitivity μg/ml *1	Correlation *2	Laboratory
1) 0.5mol/l tetra-n-butyl ammonium phosphate (TBA-P)					
Tokyo Chemical	8.2	**good**	0.02	0.999	1, 2, 3
Wako Pure Chemical	3.4	no peak	-	-	1, 2
	7.5 *3	**not clear**	2	-	2
GL Sciences	8.8	**not clear**	5	-	1
Nacalai Tesque	8.4	**not clear**	2	-	1
Acros Organics	3.8	no peak	-	-	2
2) 0.25mol/l tetra-n-butyl ammonium sulfate *4					
Waters	7.2	**not clear**	1	0.984	2

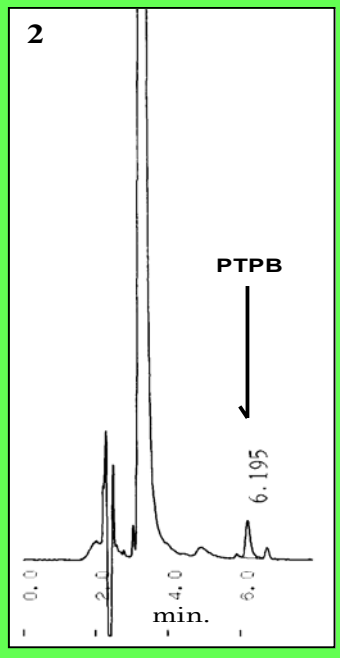
*1 Detectable minimum concentration of PTPB
*2 Correlation coefficient of calibration curve
*3 The pH of mobile phase was adjusted to 7.5
*4 Mobile phase: acetonitrile/water/ion-pair reagent (60/38/2(v/v/v))
Laboratory
1.Hokko Chemical Ind. Co., Ltd.
2. Chugoku Marine Paints Co., Ltd.
3. Kanae Paint Co., Ltd.

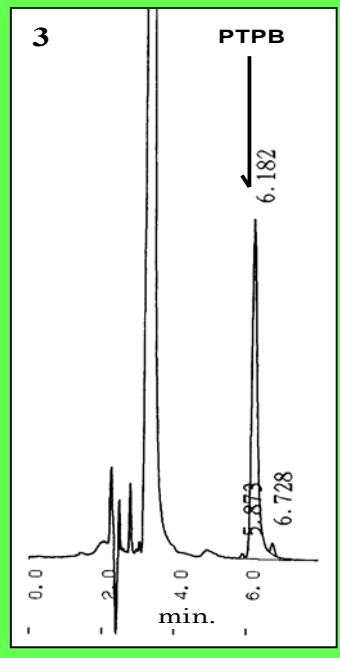
Ion-pair reagent (1)

The chromatogram of PTPB under the typical conditions



1.
No TBA-P
Mobile phase:
Acetonitrile/water
(67/33)



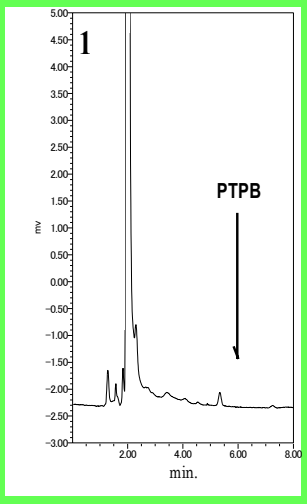


2. and **3.**
TBA-P: Tokyo Chemical
Mobile phase:
Acetonitrile/water/TBA-P (67/32/1)
PTPB: **2.** 0.1 μg/ml **3.** 1μg/ml

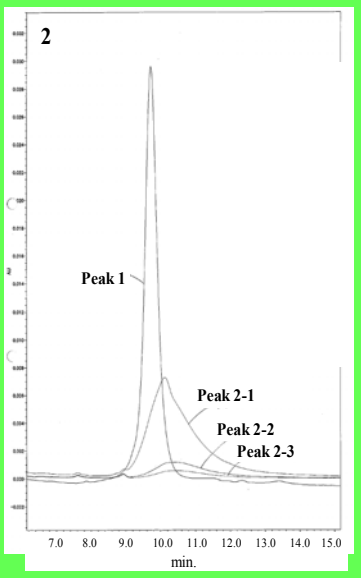
Apparatus: Hitachi L-7000
Column: Inertsil ODS-3(4.6x250mm, 5um)
Flow rate: 1ml/min.
UV detector: 220nm
Injection volume: 10μl

Ion-pair reagent (2)

The chromatograms of PTPB and TBA-P

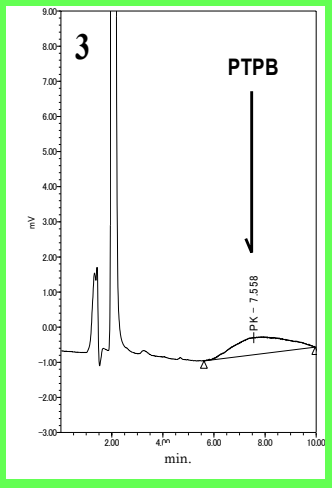


1. TBA-P:
Wako Pure Chemical
Acetonitrile/water/TAB-P
(67/32/1), without adjusting pH
PTPB 20 μg/ml

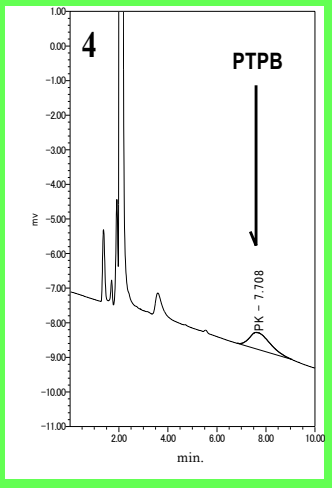


2.
Peak1
TBA-P: Tokyo Chemical
Acetonitrile/water/TBA-P
(65/34/1)
PTPB 20 μg/ml

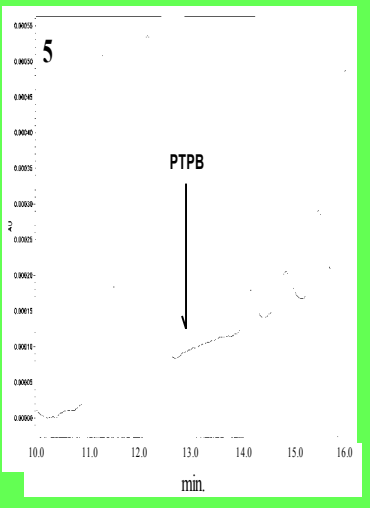
Peak2-1, 2-2 and 2-3
TBA-P: Wako Pure Chemical
Acetonitrile/water/TBA-P
(65/33/2), adjusted to pH7.5
Peak2-1=PTPB 20 μg/ml
Peak2-2=PTPB 4 μg/ml
Peak2-3=PTPB 2 μg/ml



3. TBA-P:
GL Sciences
PTPB 5 μg/ml



4. TBA-P:
Nacalai Tesque
PTPB 2 μg/ml



5. TBA-P:
Acros Organics
PTPB 20 μg/ml

HPLC column: Inertsil ODS-3

HPLC column

Table 2) The Shape of PTPB Peak and reversed-phase HLPC columns

Product I.D.x length, particle size		Supplier	Shape of PTPB peak	Sensitivity µg/ml *1	Correlation *2	t_R, min. *3	Laboratory *4
Inertsil ODS-3	4.6x250mm, 5um	GL Sciences	**good**	0.02	0.999	8.2 [1)]	1, 2
						6.2 [2)]	1
Wakosil 5C18	4.0x200mm, 5um	Wako Pure Chemical	**good**	0.02	0.999	6.1 [2)]	1
Mightysil RP-18	4.6x250mm, 5um	Kanto Chemical	**good**	0.02	0.999	6.1 [2)]	1
X-Terra C18	4.6x150mm, 3um	Waters	**good**	0.02	-	3.5 [1)]	1
Atlantis C18	2.1x150mm, 3um	Waters	**good**	0.02	-	2.5 [1)]	1
Nova Pak C18	4.6x150mm, 4um	Waters	**good**	0.02	-	3.8 [3)]	1
L-Column C8	4.6x150mm, 5um	CERI	**good**	0.02	0.999	4.8 [1)]	3
SunFire C18	4.6x150mm, 5um	Waters	not clear	2	-	5.2 [1)]	1

*1 Detectable minimum concentration of PTPB

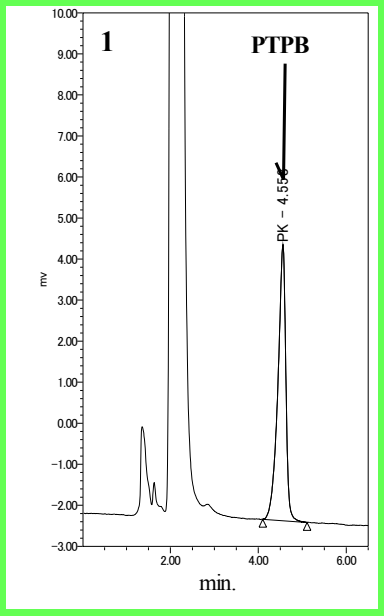
*2 Correlation coefficient of calibration curve

*3 t_R: Retention time
Mobile phase:
1) acetonitrile/water/TBA-P 65/34/1(v/v/v),
2) acetonitrile/water/TBA-P 67/32/1
3) acetonitrile/water/TBA-P 70/29/1
TBA-P: Tokyo Chemical

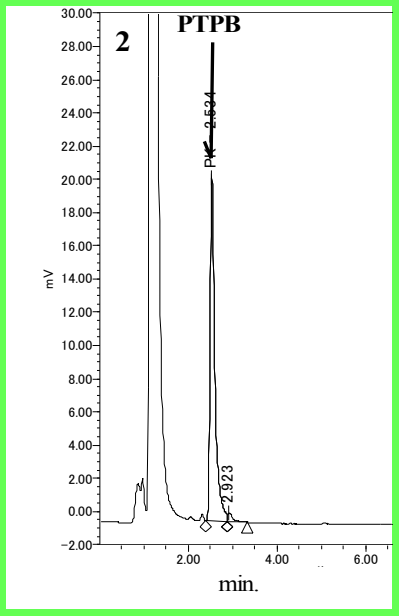
*4 Laboratory 1:Hokko Chemical, 2: Chugoku Marine Paints, 3: Kanae Paint

HPLC column

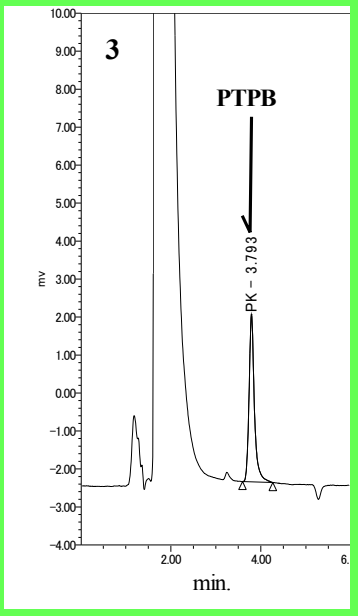
The chromatograms of PTPB and reversed-phase HPLC columns



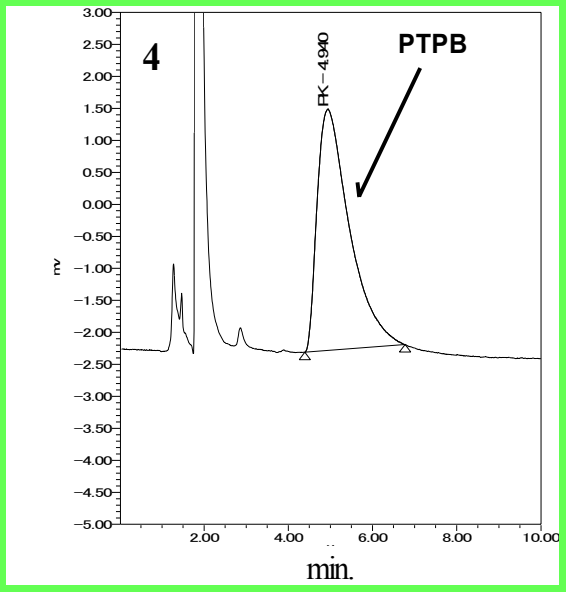
1. Wakosil 5C18
PTPB 2 μg/ ml



2. Atlantis C18
PTPB 2 μg/ml



3. Nova-PakC18
PTPB 2 μg/ml



4. SunFire C18
PTPB 5 μg/ml

TBA-P: Tokyo Chemical

Conclusion

The clear and sharp peak of PTPB was obtained by using

1. Ion-pair reagent:
   0.5mol/l tetra-n-butyl ammonium phosphate (TBA-P)
   supplied by Tokyo Chemical

2. HPLC column: commonly used ODS column
   except SunFire™ (Waters)

REFERENCES

1) ISO 15181-1:2007, Determination of release rate of biocides from antifouling paints – Part 1: General method for extraction of biocides
2) ISO/FDIS 15181-4:2008, Determination of release rate of biocides from antifouling paints – Part 4: Determination of pyridine-triphenylborane(PTPB) concentration in the extract and calculation of release rate
3) K. Takahashi, E. Yoshikawa, M. Akiyama, K. Kitaori and S. Masuoka: J. Jpn. Soc. Colour Mater., 78(2), 50-57(2005)