## TECHNICAL NOTE

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# Screening of Pharmaceuticals and Drugs in Synovial Fluid of the Knee Joint and in Vitreous Humor by Fluorescence Polarization Immunoassay (FPIA)

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**ABSTRACT:** The investigations presented are aimed at testing whether ABBOTT's fluorescence polarization immunoassays for identification of drugs of abuse and pharmaceuticals in serum and urine are applicable to knee joint synovial fluid and vitreous humor. The survey confirms the general applicability of the tests to the respective body fluids without previous precipitation, only after liquefaction using hyaluronidase. The calculated cross-reactivities are shown in tables.

**KEYWORDS:** forensic science, forensic toxicology, fluorescence polarization immunoassay, serum, urine, vitreous humor, knee joint synovial fluid, cross-reactivities, substance abuse detection, pharmaceuticals

The diagnostic FPIA tests offered by Abbott GmbH for determination of the presence of pharmaceuticals or drugs in serum samples are not to be recommended for other investigated material without further testing (1). In the literature, the application of test kits specially developed for serum on urine and vice versa (2,3), stomach content and gallbladder (1) as well as both test kits on stomach content and gall-bladder (1) has been described.

For the clarification of cause of death under already advanced autolysis and decay of postmortem obtainable blood and urine, synovial fluid of the knee joint and vitreous humor make possible a rapid screening of toxicologically relevant pharmaceuticals or groups thereof. They are easily obtainable and the process of decay begins relatively late (4–15).

Studies of the application of FPIA urine and serum tests to knee joint fluid are not known at this time. Likewise there are only a limited number of publications available concerning the application of urine and serum tests to vitreous humor for identification of drugs pharmaceuticals or groups of pharmaceuticals (16–21), so that it seemed forensically interesting to carry out such experiments.

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### Methods

Test compounds were prepared as ethanolic solutions and stored at 4°C. Aliquots of these drug solutions were added to negative knee-joint fluid, vitreous humor and serum to give final concentrations of 100 to 100 000 ng/mL, depending upon their respective reactivity.

Vitreous humor (1 to 2 mL) was obtained by puncture from the outer corner of the eye. The synovial fluid (1 to 3 mL) was obtained by tapping. Both specimens were preserved by refrigeration at 4°C. For the analysis the vitreous humor was measured directly without deproteinization, and the knee joint fluid was measured directly following liquefaction with hyaluronidase after addition of the indicated reference materials (ng/mL or μg/mL respectively). For liquefaction of the synovial fluid, 1000 μL together with 15 μL hyaluronidase solution (5 mg hyaluronidase (EC 3.2.1.35) from taurine testes, 700 WHO-U/mg per 1 mL buffer pH 6.9) were warmed to 35°C and vortex mixed for 5 s. The phosphate buffer was preserved by a mixture of 44.8 parts of potassium hydrogen phosphate (1/15 M) and 55.2 parts of disodium hydrogen phosphate (1/15 M). The respective reference substances were mixed in thoroughly (vortex) as ethanolic solution (1 mg/1 mL). The specific relation of an antibody to its antigenes is characterized by the concept ''cross-reactivity'' (CR). Cross-reactivity (CR) is understood as follows:

$$\% \text{ CR} = \frac{\text{measured concentration} \times 100\%}{\text{added concentration}}$$

On the basis of three individual values followed the calculation of the mean values of the cross-reactivities (CR).

### Results and Discussion

Table 1 demonstrates the areas of the cross-reactivities obtained for the target substances in the knee joint fluid (KF) and the vitreous humor (VH), dependent upon analyte concentration. For purposes of comparison, the cross-reactivities of the analytes in urine (U) and serum (SE) given in the directions for use of the test are also included.

As is generally known, there can be lot to lot differences in the antibody in the kit which lie within the bounds of the measurement system. The manufacturer takes account of these by listing corresponding variables for the respective control samples of the calibrator substance. Therefore only deviations clearly beyond the respective CR values given by Abbott will be discussed.

TABLE 1—*Cross reactivity: pharmaceuticals.*

| Substance Name | CR (%) KF | VH | SE (ABBOTT) | ng/mL |
|---|---|---|---|---|
| Tricyclic Antidepressants | | | | |
| Amitriptyline | 141 – 111 | 142 – 90 | 80 – 91 | 100 – 500 |
| Amitriptyline-oxide | 134 – 107 | 110 – 68 | 95 – 73* | 100 – 500 |
| Clomipramine | 127 – 61 | 128 – 47 | 51 – 41 | 100 – 500 |
| Desipramine | 177 – 107 | 182 – 111 | 90 – 87 | 100 – 500 |
| Doxepin | 60 – 40 | 74 – 32 | 42 – 32 | 100 – 500 |
| Imipramine | 170 – 122 | 173 – 111 | Calibrator | 100 – 500 |
| Nortriptyline | 160 – 94 | 96 – 67 | 97 – 81 | 100 – 500 |
| Noxiptiline | 60 – 34 | 73 – 34 | 46 – 29* | 100 – 500 |
| Trimipramine | 110 – 65 | 80 – 56 | 67 – 55 | 100 – 500 |
| Anticonvulsants | | | | μg/ml |
| Carbamazepine | 101 – 91 | 105 – 95 | Calibrator | 2.0 – 20 |
| Phenytoin | 111 – 104 | 110 – 96 | Calibrator | 2.5 – 40 |
| Primidone | 101 – 103 | 121 – 88 | Calibrator | 2.0 – 24 |
| Ethosuximide | 99 – 96 | 96 – 92 | Calibrator | 10 – 150 |
| Analgesics | | | | μg/ml |
| Salicylates | | | | |
| Salicyl-Acid | 104 – 91 | 136 – 101 | Calibrator | 50 – 700 |
| Acetylsalicyl-Acid | 12 – 8 | 18 – 6 | 0 – 1.6 | 50 – 1000 |
| Salicylamide | 0 | 18 – 1 | 0* | 50 – 1000 |
| Benzoic-Acid | 0 – 1.5 | 16 – 1 | 0 – 0.8 | 50 – 1000 |
| Paracetamol | 86 – 95 | 100 – 94 | Calibrator | 10 – 200 |

*Own investigation

In Table 1 the cross-reactivities of tricyclic antidepressants, anticonvulsants and analgesics in serum, knee joint fluid and vitreous humor were compared with the serum test from Abbott. In comparison to serum, a greater cross-reactivity of tricyclic antidepressants in low concentrations (with the exception of doxepin and noxiptiline) was almost universally recognizable. This result applies especially to knee joint fluid, but also, with the exception of amitriptylineoxide, nortriptyline and trimipramine, to vitreous humor.

Measured cross-reactivities for individual anticonvulsants in knee joint fluid and in vitreous humor demonstrated only slight differences and accord with the cross-reactivity in serum.

The cross-reactivities of the individual benzodiazepines in knee joint fluid listed in Table 2 demonstrate only graduated differences in comparison with the respective CR rates in serum or urine. It is notable that under application of the urine test, oxazolam in urine as well as in knee joint fluid demonstrates no cross-reactivity, while cross-reactivity was demonstrated by the serum test in serum and knee joint fluid. Under the serum test an increased cross-reactivity of the knee joint fluid in comparison to cross-reactivity for alprazolam and triazolam in serum was observed, particularly in low concentrations. A reciprocal tendency occurs with chlorazepat, lormetazepam and midazolam, the cross-reactivity of which is greater in serum than in knee joint fluid. An increased cross-reactivity for chlorazepat in knee joint fluid compared to urine is recognizable with the urine test.

In Table 3 the cross-reactivities of various barbiturates, amphetamines, methamphetamines, opiates and cannabinoides as well as cocaine, benzoylecgonine, propoxyphene, phencyclidine and methadone were analyzed for the urine test. The cross-reactivity of the above-named analytes in knee joint fluid under urine testing shows a corresponding scale to that of the CR rates in urine. Increased CR rates in knee joint fluid were observed for ethylmorphine and particularly for benzoylecgonine. A decreased cross-reactivity was measured for carboxytetrahydrocannabinol with urine and vitreous humor (CR = 95 – 104% in area 10 – 100 ng/mL 4). It could be clarified experimentally that the additon of hyaluronidase for liquefaction of the knee joint fluid led in this isolated case to partial or, in small concentrations, even complete deterioration of carboxytetrahydrocannabinol, by which means the cross-reaction can be reduced to zero.

TABLE 2—*Cross reactivity: Benzodiazepines.*

| Substance Name | CR (%) KF | U (ABBOTT) | ng/mL |
|---|---|---|---|
| Benzodiazepines | | | |
| Alprazolam | 106 – 74 | 109 – 81 | 400 – 1200 |
| Bromazepam | 33 – 20 | 31 – 20 | 400 – 1200 |
| Brotizolam | 75 – 39 | 65 – 36* | 400 – 1200 |
| Chlordiazepoxide | 13 – 9 | 16 – 9 | 400 – 1200 |
| Clobazam | 38 – 27 | 47 – 28* | 400 – 1200 |
| Clonazepam | 29 – 14 | 37 – 23 | 400 – 1200 |
| Clorazepat | 77 – 76 | 44 – 40* | 400 – 1200 |
| Clotiazepam | 43 – 24 | 36 – 23* | 400 – 1200 |
| Diazepam | 132 – 124 | 133 – 143 | 400 – 1200 |
| Flunitrazepam | 50 – 30 | 52 – 35 | 400 – 1200 |
| Flurazepam | 67 – 39 | 60 – 39 | 400 – 1200 |
| Ketazolam | 106 – 102 | 93 – 90* | 400 – 1200 |
| Lorazepam | 26 – 19 | 38 – 24 | 400 – 1200 |
| Loprazolam | 30 – 16 | 21 – 15* | 400 – 1200 |
| Lormetazepam | 40 – 29 | 42 – 29* | 400 – 1200 |
| Medazepam | 90 – 70 | 92 – 67 | 400 – 1200 |
| Metaclazepam | 30 – 22 | 28 – 19* | 400 – 1200 |
| Midazolam | 85 – 48 | 87 – 62* | 400 – 1200 |
| Nitrazepam | 59 – 38 | 65 – 42 | 400 – 1200 |
| Nordiazepam | 101 – 96 | Calibrator | 400 – 1200 |
| Oxazepam | 63 – 42 | 86 – 67 | 400 – 1200 |
| Oxazolam | 0 | 0* | 400 – 1200 |
| Prazepam | 88 – 66 | 114 – 86 | 400 – 1200 |
| Temazepam | 76 – 60 | 101 – 93 | 400 – 1200 |
| Tetrazepam | 58 – 40 | 55 – 41* | 400 – 1200 |
| Triazolam | 57 – 33 | 65 – 35 | 400 – 1200 |
| | KF | SE (ABBOTT) | ng/mL |
| Alprazolam | 131 – 70 | 78 – 60 | 25 – 700 |
| Bromazepam | 10 – 6 | 14 – 0 | 75 – 700 |
| Brotizolam | 49 – 29 | 44 – 28* | 75 – 700 |
| Chlordiazepoxide | 14 – 8 | 13 – 0 | 75 – 700 |
| Clobazam | 49 – 27 | 53 – 24* | 75 – 700 |
| Clonazepam | 50 – 19 | 44 – 19 | 75 – 700 |
| Clorazepat | 32 – 22 | 113 – 39* | 75 – 700 |
| Clotiazepam | 17 – 12 | 30 – 16* | 75 – 700 |
| Diazepam | 96 – 105 | 85 – 96 | 75 – 700 |
| Flunitrazepam | 57 – 30 | 60 – 31 | 75 – 700 |
| Flurazepam | 46 – 24 | 48 – 24 | 75 – 700 |
| Ketazolam | 103 – 90 | 112 – 88* | 25 – 700 |
| Lorazepam | 63 – 17 | 40 – 22 | 75 – 700 |
| Loprazolam | 44 – 18 | 66 – 19* | 75 – 700 |
| Lormetazepam | 60 – 23 | 120 – 65* | 75 – 700 |
| Medazepam | 99 – 48 | 68 – 39 | 75 – 700 |
| Metaclazepam | 57 – 25 | 108 – 32* | 75 – 700 |
| Midazolam | 100 – 47 | 149 – 63* | 75 – 700 |
| Nitrazepam | 39 – 28 | 56 – 31 | 75 – 700 |
| Nordiazepam | 109 – 94 | Calibrator | 75 – 700 |
| Oxazepam | 62 – 35 | 76 – 40 | 75 – 700 |
| Oxazolam | 27 – 4 | 26 – 9* | 25 – 700 |
| Prazepam | 85 – 56 | 96 – 33* | 25 – 700 |
| Temazepam | 70 – 41 | 75 – 41 | 75 – 700 |
| Tetrazepam | 93 – 26 | 101 – 35* | 25 – 700 |
| Triazolam | 120 – 42 | 65 – 42 | 25 – 700 |

*own investigation.

TABLE 3—*Cross reactivity: drugs of abuse.*

| Substance Name | CR (%) KF | CR (%) U (ABBOTT) | ng/mL |
|---|---|---|---|
| Barbiturates | | | |
| Allobarbital | 30 – 25 | 31 – 29 | 400 – 1200 |
| Amobarbital | 45 – 26 | 35 – 34 | 200 – 1200 |
| Aprobarbital | 52 – 54 | 64 – 60 | 400 – 1200 |
| Barbital | 6 – 5 | 0 – 6 | 400 – 1200 |
| Butabarbital | 240 – 218 | 244 – 218 | 100 – 700 |
| Butalbital | 155 – 121 | 113 – 106 | 200 – 1200 |
| Butallylonal | 242 – 208 | 250 – 234* | 200 – 700 |
| Butobarbital | 45 – 41 | 46 – 52 | 200 – 1200 |
| Crotylbarbital | 45 – 26 | 80 – 34* | 200 – 1200 |
| Cyclobarbital | 88 – 73 | 79 – 72 | 400 – 1200 |
| Hexobarbital | 0.1 – 0.2 | 0 – 0.3 | 10000 – 50000 |
| Pentobarbital | 77 – 56 | 73 – 68 | 400 – 1200 |
| Phenobarbital | 55 – 48 | 65 – 54 | 400 – 1200 |
| Secobarbital | 135 – 101 | Calibrator | 500 – 1200 |
| Thiopental | 5 – 8 | 0 – 9 | 400 – 1200 |
| Vinylbital | 160 – 104 | 135 – 118* | 200 – 1200 |
| Amphetamine/Methamphetamine | | | |
| D-Methamphetamine | 84 – 71 | 110 – 95 | 500 – 5000 |
| D-Amphetamine | 106 – 148 | Calibrator | 500 – 5000 |
| DL-Amphetamine | 124 – 102 | 110 – 217 | 300 – 5000 |
| Propoxyphene | | | |
| Propoxyphene | 115 – 119 | Calibrator | 150 – 1200 |
| Opiates | | | |
| Codeine | 88 – 118 | 110 – 114 | 100 – 800 |
| Dihydrocodeine | 44 – 36 | 64 – 53 | 300 – 1000 |
| Ethylmorphine | 147 – 128 | 55 – 86 | 100 – 500 |
| Hydrocodone | 37 – 29 | 58 – 46 | 300 – 1000 |
| Morphine | 93 – 100 | Calibrator | 100 – 800 |
| Oxycodone | 23 – 10 | 23 – 10 | 200 – 1000 |
| Pethidine | 7 – 3 | 9 – 3* | 300 – 1000 |
| Cocaine-Metabolite | | | |
| Cocaine | 2.5 – 2.1 | 0 – 0.8 | 1000 – 100000 |
| Benzoylecgonine | 235 – 168 | Calibrator | 200 – 1000 |
| Cannabinoides | | | |
| $\Delta^6$-Tetrahydrocannabinol | 0 – 26 | 0 – 9* | 10 – 400 |
| $\Delta^9$-Tetrahydrocannabinol | 0 – 20 | 0 – 8* | 10 – 400 |
| Carboxytetrahydrocannabinol | 0 – 71 | Calibrator | 10 – 100 |
| Phencyclidine I | | | |
| Phencyclidine | 89 – 104 | Calibrator | 50 – 400 |
| Methadone | | | |
| Methadone | 117 – 98 | Calibrator | 200 – 3000 |

*Own investigation.

## Conclusion

Both fluid specimens make possible a satisfactory screening for all individual compounds examined. However, a relevant general increase in cross-reactivity either in the case of vitreous humor or of knee joint synovial fluid in comparison with serum or urine cannot be established.

It is known that a high cross-reactivity means that the presence of even very small amounts of substance from the test system will be detected; conversely, a low cross reactivity indicates that only larger amounts of substance result in a positive test result.

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